

THE NEURONAL ORGANISATION
OF STRIDULATION AND FLIGHT IN THE CRICKET,
TELEOGRYLLUS COMMODUS

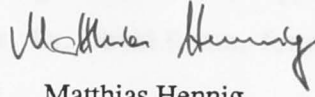
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Declaration

The work presented in this thesis is my own.



Matthias Hennig

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SUMMARY

Have you seen the snow leopard ?

No! Isn't that wonderful ?

Peter Matthiessen, 1979. The Snow Leopard.

SUMMARY

A male cricket, *Teleogryllus commodus* (Walker), uses his wings during both stridulation and flight. During stridulation, the raised forewings are rubbed against each other and this motor pattern switches repeatedly between a slow chirp (~ 20 Hz) and a fast trill (~ 35 Hz). During flight, both pairs of wings are unfolded and move regularly up and down (~ 35 Hz). The neuronal basis for these two motor patterns, which originate in the meso- and metathoracic ganglia, was examined in order to describe similarities and differences in the mechanisms of motor pattern generation, and to determine the overlap and interaction between these two neuronal circuits. Electromyograms, extracellular nerve recording, and intracellular recording and dye filling techniques were used. Stridulation was elicited routinely in intact and deafferented crickets by placing a suction electrode on the brain and passing a constant DC-current, while flight was evoked by brief wind puffs directed at the head or the cerci.

Both motor patterns are generated with similar cycle periods in intact crickets. The stridulatory motor pattern changes little after the removal of peripheral feedback, but during flight a distinct increase in cycle period is observed after deafferentation.

The burst pattern of motoneurons during stridulation and flight is shaped by both excitatory and inhibitory inputs and, as judged on the basis of distinct synaptic phases, the synaptic input to motoneurons during stridulation has some common elements during the chirp and the trill, despite the change in rhythm. The input to the same motoneurons during flight, however, appears to be entirely different. Motoneurons innervating hindwing muscles are only active during flight.

Identified interneurons form two pools, one active during stridulation, the other active during flight. Stridulatory interneurons were active during both the chirp and the trill, supporting the idea of common synaptic inputs to motoneurons during the expression of these two rhythms. Some flight interneurons were able to reset the flight rhythm, and one is likely to initiate it. Characteristics of the motor patterns, and the activity of motoneurons and interneurons, suggest that the mechanisms of initiation and pattern generation are different for stridulation and flight.

Additional evidence that the circuits for stridulation and flight are also independently organised comes from the observation that both motor patterns may be elicited and expressed simultaneously, as has been observed in both motoneuronal and interneuronal recordings. Under these circumstances no phase coupling, as might be expected if a single oscillator generated both rhythms, has been observed.

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As the "... mechanisms for the coding, transformation and storage of quantitative information seem to be intimately associated with ... living matter" (Rader 1961), the nervous system is the means by which an animal perceives the physical world, generates coordinated movements, and memorises the past. The study of the neuronal mechanisms of sensory perception, motor control and learning falls within the scope of neuroethology (Oliver and Mauk 1983). Some of the questions and approaches in neuroethology have a long history. For instance, Sherrington (1906) described reflexes as a series of reflexes (or chains), and also Friedlander (1934). He proposed that sequential activation of sensory reflexes (i.e. chain reflexes) is the basis for the generation of a motor rhythm. A chain reflex is defined as a reflex in which the activation of one reflex leads to the activation of another reflex (Friedlander 1934). This view was later modified by Brown (1934, v. Hater 1937) and the sensory feedback only serves to adjust for perturbations during the execution of the motor program. These two concepts are still very much alive in neuroethological research today (Schwartz 1980, Pearson 1985, Gazing 1986) and as often in biology, they are not mutually exclusive. This was recently demonstrated for the neural organisation of flight behaviour in an insect, the locust, where a centrally generated rhythm is integrated with reflex information from the wings to produce the coordinated behaviour (Wolf and Pearson 1983, 1985).

Sherrington's (1906) idea of a final common path consisting of neurons which may be activated by several opposing reflexes leads to another area of neuroethological interest today. How does the nervous system organise different behaviours which use the same limb and therefore the same muscles? To answer this question Sherrington studied the scratch and paw withdrawal behaviours of dogs and proposed a reciprocal inhibition between

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As the "... mechanisms for the coding, transformation and storage of information seem to be inevitably associated with ... living matter" (Roeder 1963), the nervous system is the means by which an animal perceives the physical world, generates coordinated movements, and memorises the past. The study of the neuronal mechanisms of sensory perception, motor control and learning falls within the scope of neuroethology (Huber and Markl 1983). Some of the questions and concepts in neuroethology have a long history. For instance, Sherrington (1906) described behaviour as a series of reflexes (reflex chains, see also Friedländer 1894). He proposed that sequential activation of sensory reflexes (i.e. each phase of a movement exciting peripheral sensory receptors which then activate the following phase of the movement and so on...) is the basis for the generation of a motor rhythm. A different view held that pattern (or rhythm) generation takes place entirely within the nervous system (Brown 1914, v.Holst 1937) and that sensory feedback only serves to adjust for perturbations during the execution of the motor program. These two concepts are still very much alive in neuroethological research today (Selverston 1980, Pearson 1985, Getting 1986) and as often in biology, they are not mutually exclusive. This was recently demonstrated for the neural organisation of flight behaviour in an insect, the locust, where a centrally generated rhythm is integrated with reflex information from the wings to produce the coordinated behaviour (Wolf and Pearson 1988, 1989).

Sherrington's (1906) idea of a final common path consisting of muscles which may be activated by several opposing reflexes leads to another area of neuroethological interest today. How does the nervous system organise different behaviours which use the same limb, and therefore the same muscles? To answer this question Sherrington studied the scratch and paw withdrawal behaviours of dogs and proposed a recurrent reciprocal inhibition between

interneuronal circuits which compete over the final common path. Questions regarding the neuronal organisation underlying different motor patterns which activate the same muscles still generate great interest, involving cellular investigations in both vertebrates (Berkinblit et al. 1987, Carter and Smith 1986, Robertson et al. 1985), and invertebrates (Getting and Dekin 1985, Heitler 1985, Ramirez and Pearson 1988).

This is the broad background against which the current study is set. How does the nervous system generate and execute a given behaviour and how are different behaviours which activate the same limb or appendage coordinated? For my approach to these questions I have chosen the cricket *Teleogryllus commodus* (Walker) as an experimental preparation. A cricket such as *T.commodus* activates its wings during the flight and singing behaviours, therefore allowing the neuronal organisation of both behaviours to be compared. Furthermore, the flight and stridulation behaviours have been extensively studied previously which is an important advantage in exploring nervous function.

1. Cricket flight and singing behaviours

Ethological and neurobiological research on cricket behaviour has long contributed to our understanding of nervous function (for a summary of the extensive research see Huber et al. (1989)). Investigation of the communication behaviour of crickets dates back to the turn of this century (Regen 1913) and the investigation of the neuronal basis of singing and phonotactic behaviour continues today. Huber's groundbreaking work during the 1960's on the different forms of behaviour and their neuronal control established the cricket as a model animal in neurobiological and neuroethological research offering all of the experimental advantages of an invertebrate preparation and allowing us to address important issues in neuroethology. Among these issues are the neural mechanism of acoustic pattern recognition; the neuronal organisation of flight behaviour, which may involve integration of acoustic information, and resultant positive or negative phonotactic steering; and the mechanisms of central pattern generation during flight and singing, their descending and peripheral control, and their development. In this introduction I provide a brief summary of what is known regarding the neuronal organisation of stridulation and flight in the cricket, *Teleogryllus commodus* and the thoracic neuromuscular system that produces them.

1.1. Neuromuscular organisation of the mesothoracic segment of the cricket

The forewings, which are activated during stridulation and flight (Fig. 1.1), insert on the mesothoracic segment of the cricket and connect to the thorax via a number of sclerites. The forewing itself is a sclerotized structure with a special morphology adapted for efficient sound production (Nocke 1971). Due to the morphological design of the wing hinge, the forewing has two positions around which it may rotate: (1) a raised position employed for the inward and outward movement during stridulation (Fig. 1.1 A), and (2), an outward position employed for the up and down movement during flight (Fig. 1.1 B; Bentley and Kutsch 1966, Kutsch 1969). The wing and the wing hinge are innervated by receptors and sensory organs which monitor the wing position and movements such as the stretch receptor, the chordotonal organ, the tegula, campaniform sensilla, and mechanoreceptive hairs; all of which may play important roles in stridulation and flight (Huber 1963, Kutsch and Huber 1970, Möss 1971, Elliott and Koch 1983, Schöffner and Koch 1987). The wing is moved by direct as well as indirect muscles as described in detail by Kutsch (1969). There is a distal layer of muscles (98, 2nd basalar; 99, subalar) which depress the wing during flight and open the wing during stridulation and a proximal layer of muscles (89, promotor coxae; 90, remotor coxae) which elevate the wing during flight and close the wing during stridulation (Fig. 1.1 A, B; Huber 1960a, Bentley and Kutsch 1966, Bentley 1969a, 1969b, Kutsch 1969). The dorsal longitudinal muscle acts as an indirect depressor during flight, but does not function as a single entity during stridulation (Kutsch 1969). Each mesothoracic wing muscle is innervated by from 2 - 5 motoneurons (Kutsch 1969, Elepfandt 1980, Elliott 1983, Wang and Robertson 1989). The organisation of the metathoracic segment is similar to that of the mesothoracic segment. However, the hindwings are active only during flight and not during stridulation (Kutsch 1969). During stridulation each closing movement of the forewings generates a sound pulse and the fast repetition of opening and closing strokes results in a calling song with a species-specific sound pattern. The calling song of *Teleogryllus commodus* contains two different temporal rhythms, the chirp and the trill, which alternate (Fig. 1.1 A; Bentley and Hoy 1972). The "slow" chirp rhythm is immediately followed by the "fast" trill. In flight both pairs of wings unfold and beat rhythmically up and down, so that muscles which act as openers/closers during stridulation depress/elevate the wings during flight (Fig. 1.1 A, B).

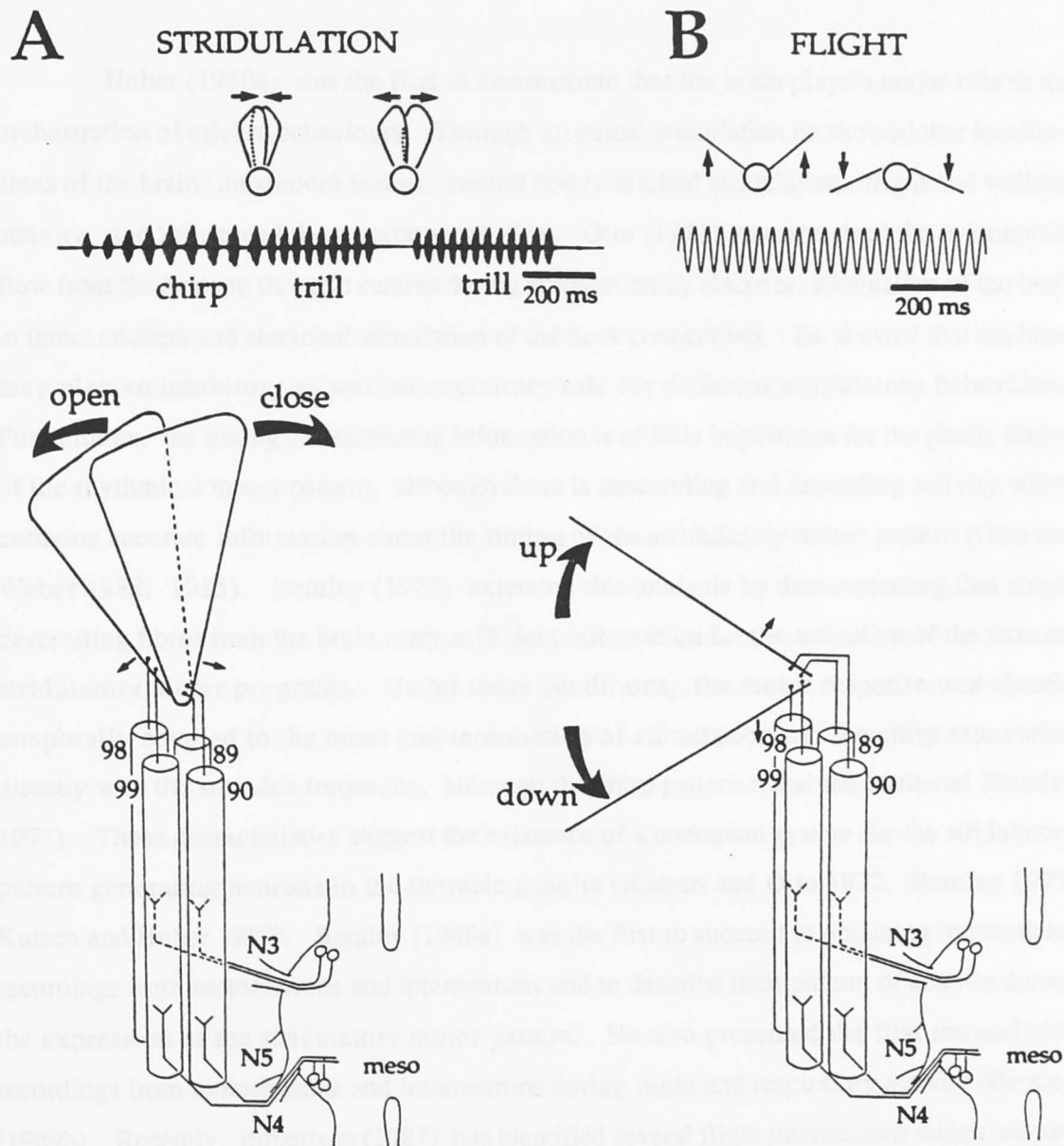


Fig. 1.1 A, B. Sketch of the neuromuscular organisation of the mesothoracic segment of the cricket. **A** During stridulation, repeated closing movements of the forewings generate the species-specific calling song of *Teleogryllus commodus*, during which the motor pattern switches between the chirp and the trill. The forewings are opened by muscles 98 and 99 and closed by muscles 89 and 90. **B** During flight, the wings move regularly up and down. The forewings are moved by the same major muscles which are active during stridulation (**A**). Muscles 98 and 99 depress the wings, whereas muscles 89 and 90 elevate them.

1.2. Neuronal organisation of stridulation and flight

Huber (1960a) was the first to demonstrate that the brain plays a major role in the orchestration of cricket behaviours. Through electrical stimulation he showed that localised areas of the brain (mushroom bodies, central body) elicited stridulation, flight and walking activity, and influenced the respiratory rhythm. Otto (1971) characterised the information flow from the brain to thoracic centres during stridulation by electrical stimulation of the brain in intact crickets and electrical stimulation of the neck connectives. He showed that the brain may play an inhibitory as well as excitatory role for different stridulatory behaviours. Furthermore, the timing of descending information is of little importance for the phasic timing of the rhythmical motor pattern, although there is descending and ascending activity which contains accurate information about the timing of the stridulatory motor pattern (Otto and Weber 1982, 1985). Bentley (1977) extended this analysis by demonstrating that single descending fibres from the brain carry sufficient information for the activation of the thoracic stridulatory motor programs. Under these conditions, the motor response was closely temporally coupled to the onset and termination of stimulation and the chirp rate varied directly with the stimulus frequency, although the chirp pattern remained unaltered (Bentley 1977). These characteristics suggest the existence of a command system for the stridulatory pattern generating neurons in the thoracic ganglia (Kutsch and Otto 1972, Bentley 1977, Kutsch and Huber 1989). Bentley (1969a) was the first to succeed in obtaining intracellular recordings from motoneurons and interneurons and to describe their pattern of activity during the expression of the stridulatory motor pattern. He also presented the first intracellular recordings from motoneurons and interneurons during flight and respiratory activity (Bentley (1969b). Recently, Robertson (1987) has identified several flight interneurons which are part of the central oscillator, as evidenced by their ability to reset the flight motor pattern permanently.

Huber's (1960a) studies of the stridulation and flight behaviours showed important similarities between the two motor patterns and this gave rise to the idea that neuronal circuitry, and possibly even a central oscillator, may be shared between the two behaviours. This view fitted in with the proposed common evolutionary origin of stridulation and flight (Zeuner 1930, Huber 1962, Alexander 1962) and received support from subsequent studies by Bentley and Kutsch (1966), Kutsch (1969), Bentley (1969b), and Möss (1971). Kutsch (1969) further suggested that flight, stridulation, walking, and respiration may be driven by

two oscillators, one fast and the other slow. However, these suggestions were drawn largely on the basis of electromyographic analysis, and evidence on the mechanisms of pattern generation via intracellular recordings from interneurons during the actual expression of both motor patterns was not obtained.

Thus, the state of knowledge at the beginning of this study may be summarised as follows: (1) the stridulatory and flight motor patterns activate the same sets of muscles which insert on the forewing; (2) the pattern-generating networks for both behaviours are located in the thoracic ganglia and their expression is orchestrated by the brain; (3) a considerable overlap of neuronal networks for motor pattern generation seems likely, however, evidence for this from interneuronal recordings is not yet available.

1.3. *Relevant concepts in motor control*

This thesis investigates the neuronal organisation underlying motor pattern generation during cricket flight and stridulation. What are some of the principles in neuronal organisation of motor systems that have been found to underlie other invertebrate behaviours and that may be of relevance here? A useful and reductionist approach to the neuronal organisation underlying a behaviour is its description as subsystems with certain motor tasks. Roughly, these subsystems may be grouped as (1) the initiating sensory receptors, (2) the command system, (3) the pattern generator network, and (4) the motoneuronal and muscular output. Under this scheme subsystems (2) and (3) offer the best prospect for deriving common principles.

Command system: Following the original use of the term "command neuron" or "initiating system" by Wiersma and Ikeda (1964), there have been numerous attempts to define these concepts precisely (for discussions of this concept see Kupfermann and Weiss 1978, 1986, Stein 1978, DiDomenico and Eaton 1988). According to Kupfermann and Weiss (1978) a neuron may be considered to be a command neuron for a particular motor pattern if (1) this neuron is active during the motor pattern, (2) depolarising current injection elicits the motor pattern (sufficient criterion), and (3) hyperpolarising current injection prevents the motor pattern (necessary criterion). The application of those criteria proved to be very difficult in practice, since often neurons fulfilled criteria (1) and (2), but not (3). This failure was often due to the observation that several neurons act in concert to elicit or to

suppress a motor pattern (leech swimming: Nusbaum et al. 1987; locust flight: Pearson et al. 1985), but usually only the activity of one interneuron could be influenced at a time.

Command neurons can be divided into several categories according to their type of activity (Stein 1978). (1) Gating neurons are tonically active during the expression of a motor pattern, and often provide a direct drive to the pattern generating circuitry which then generates a rhythmical motor output (Kristan and Weeks 1983, Pearson et al. 1985, Nusbaum et al. 1987). (2) Trigger neurons generate a brief burst upon sensory stimulation and are active only at the beginning of a motor sequence. These neurons are known to generate a prolonged excitation in other command neurons (leech) and pattern generating neurons (Roberts et al. 1983, Brodfuehrer and Friesen 1986). (3) Oscillatory neurons show rhythmical bursting in phase with the motor pattern bursts and may feed phasic information into the motor pattern generating circuitry or they may also be considered part of that circuitry (lobster stomatogastric rhythm: Robertson and Moulins 1981, 1984; Selverston and Moulins 1987; *Tritonia* swimming: Getting 1983, Getting and Dekin 1985; gastropod feeding: Davis 1977, Benjamin 1983, Benjamin and Elliott 1989).

There is some evidence to suggest that in the cricket a command system of the gating type controls stridulatory activity (Otto 1971, Bentley 1977). Gating neurons have also been described for the initiation of locust flight (Pearson et al. 1985, Ramirez 1988), however, here similarities with the cricket flight system with respect to flight initiation are still speculative.

Motor pattern generation: Command systems commonly drive a motor pattern generator consisting of a set of central neurons which show oscillations in membrane potential in phase with the motor pattern. These neurons may be grouped into (a) neurons which determine the frequency of the motor rhythm as a result of their synaptic connections and interactions (i.e. they constitute the central oscillator, Grillner 1977), and (b) neurons which help to shape the motoneuronal burst for appropriate timing of muscle activity without influencing the timing of the whole motor program. Members of the first group, which may also contain motoneurons (Selverston and Moulins 1987, Friesen 1989) and peripheral elements (Wendler 1974, Reye and Pearson 1988, Wolf and Pearson 1988), are recognised by (1) their oscillations in membrane potential which are phase-locked to the motor bursts, and (2) their capacity for resetting the motor rhythm on injection of a brief and physiologically relevant (i.e. shorter than the cycle period) current pulse during an experimental recording (Pinsker and Ayers 1983). The mechanisms for rhythm generation

may be divided into three groups (Getting 1988, Pearson 1986), the most common of these possibly being recurrent inhibition and delayed excitation which have been described for several motor systems (leech swimming: Friesen 1989; *Tritonia* swimming: Getting 1989b; locust flight: Robertson and Pearson 1985; gastropod feeding: Benjamin and Elliott 1989). In the cricket, the existence of such resetter interneurons has been shown for the flight system (Robertson 1987), but not for the stridulatory system. It is, however, likely that such interneurons exist in the thoracic nerve cord, since the thoracic ganglia are able to generate an almost normal stridulatory pattern from an unpatterned drive (i.e. by gating) from descending connectives (Kutsch and Otto 1972, Bentley 1977).

Peripheral feedback from proprioceptors to the pattern generator: The ability of the reduced nervous system to produce a central rhythm which is similar to the intact motor pattern can be demonstrated for many rhythmic behaviours (i.e. central pattern generation, Wilson 1961, Delcomyn 1980, Grillner 1985). On the other hand, peripheral information may have strong influences on the central timing of muscular, motoneuronal, and interneuronal burst patterns (locust flight: Pearson et al. 1983, Pearson and Wolf 1987, Wolf and Pearson 1988; stick insect walking: Bässler 1983; cat: Koshland and Smith 1989; chicken: Bekoff 1987). These findings raise some doubt about the usefulness of the concept of central pattern generation as the basis for the generation of a motor rhythm (Pearson 1985). However, there is little dispute about central and peripheral interactions underlying almost all motor acts, although behaviours may differ in the weighting of these interactions in order to make the movement best adapted for its task. During stridulation in the cricket, peripheral influences appear to affect wing position, wing movement amplitude, and mutual wing pressure (Kutsch and Huber 1970, Schöffner and Koch 1987). However, the timing of the motor bursts appears to be independent of peripheral feedback. The cricket flight system was initially reported to be unaffected by the removal of peripheral information (Bentley 1969b, Möss 1971), but Robertson (1987) has recently demonstrated changes upon deafferentation.

Switching motor patterns: Exposed to changing environmental situations, an animal must make decisions about how to behave next, that is, whether to continue or to terminate the current behaviour, to go into a quiescent state, or to activate a new or additional behaviour program. Often there may be an overlap in the musculature activated by different behaviours as, for instance, during walking and scratching (cat: Berkinblit et al 1978; dog: Sherrington 1906; turtle: Robertson et al. 1985; chicken: Bekoff 1986), during jumping, kicking, and swimming (locust: Pflüger and Burrows 1978), during ingestion and egestion (*Aplysia*: Croll

and Davis 1987), or during singing and flight behaviour in crickets (Huber 1962, Kutsch 1969). If one views a motor system as composed of neuronal subsystems with certain tasks, questions regarding the separation, overlap, and sharing of neuronal function arise, as do questions concerning the mechanisms of switching between motor patterns. Currently, there is experimental evidence for two different forms of neuronal organisation which apparently form the extremes of the a spectrum. Firstly, there may be two separate networks which generate their rhythms independently of one another, but their outputs converge on the same final pathway. For example, Ramirez and Pearson (1988) recently investigated the neuronal basis for the bifunctionality of thoracic muscles in the locust which serve to activate the leg during walking and the wing during flight (Wilson 1962). They suggested that there may be separate networks for the generation of each motor pattern, since both motor patterns could be elicited simultaneously, and interneurons formed two distinct pools with the activity of each pool being restricted to the expression of its respective motor pattern. Secondly, there may be one multifunctional or polymorphic network which is capable of operating in different modes in order to generate different behaviours (Getting 1989a). For example, the sea slug *Tritonia* escapes from a predator (e.g. a starfish) by an initial reflex withdrawal upon contact and a series of undulatory swimming movements. Both the withdrawal and swimming behaviours are mediated by the same sets of motoneurons and dorsal and ventral muscles (Getting 1989b). Getting and his coworkers were able to demonstrate that there is one interneuronal network which can account for both behaviours (reviewed in: Getting 1983, Getting and Dekin 1985, Getting 1989b). A circuit is moulded out of the network and this involves some interneurons which are active in both motor patterns, whereas others are restricted to one. Such a network is usually not capable of generating both motor patterns at the same time.

The cricket is an ideal preparation for investigating the neuronal organisation underlying different behaviours, since the forewings are involved in both stridulation and flight. Thus this thesis first seeks to determine the principal similarities and differences in neuronal organisation during these two activities and then to describe the neuronal interactions which allow the nervous system to activate the same appendage in different ways during the two behaviours. The cricket is well suited for such an investigation because both behaviours are stereotyped and may readily be elicited under experimental conditions. In addition, the nervous system is easily accessible for electromyographic and extracellular recording and stimulating techniques as well as for intracellular recording and cell identification. Chapters 2 and 3, respectively, investigate the neuronal basis for the stridulation and for the flight

behaviours by characterising the motor patterns in intact and deafferented animals, by recording intracellularly from motoneurons innervating wing muscles, and by recording intracellularly from interneurons. Chapter 4 investigates the neuronal mechanisms by which the same appendage is activated during the two behaviours. Chapter 5 discusses similarities and differences in the motor pattern generation for each behaviour based on the results of chapters 2 and 3 and presents the findings of chapter 4 in the context of current research on other systems.

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CHAPTER 2

NEUROMUSCULAR ACTIVITY DURING STRIDULATION
IN THE CRICKET, *TELEOGRYLLUS COMMODUS*

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1. SUMMARY

The central nervous control of singing behaviour in male crickets (*Teleogryllus commodus*) has been investigated using electromyograms (EMG) and intracellular recording techniques. A preparation is described in which the species-specific stridulatory behaviour can be elicited with the normal chirp and trill pattern. Stridulation was induced routinely in intact crickets as well as in crickets prepared for intracellular analysis (Fig. 2.1).

1. EMG recordings were taken from opener muscle 99 (subalar) and closer muscle 90 (remotor coxae) of intact singing animals. The intervals between bursts in both opener and closer muscles vary with cycle duration (opener to opener) and maintain their phase constancy. During the chirp the closer muscle activity (burst duration, number of spikes per burst) increases considerably as compared with the trill, but opener muscle activity remains unchanged (Fig. 2.2).

2. The preparation necessary for the intracellular analysis of motor patterns involved deafferentation of the thoracic ganglia, in which the stridulatory pattern generator resides. This resulted in an increase of the period duration of the chirp and trill by about 20 % (Fig. 2.3). Otherwise, only minor changes in the motor pattern were noted after deafferentation except for a marked increase in opener burst duration (Fig. 2.3), which did not affect the phase relationship described for the intact preparation.

3. The investigation of synaptic input to identified motoneurons 99 (opener) and 90 (closer) revealed differences in the burst patterns in these neurons during the chirp as compared with the trill, but also some common synaptic components (Figs. 2.6 and 2.7). This suggests that some interneurons are activated during the chirp and trill, whereas others are only activated during the chirp.

4. Seven interneurons which were rhythmically active during stridulation are newly described by recording intracellularly in the posterior half of the metathoracic ganglion (Fig. 2.8). All of these interneurons were active during both the chirp and the trill, and they showed changes in their burst patterns during these different phases of the song (Fig. 2.9).

2. INTRODUCTION

The stridulatory behaviour of male crickets is well suited to investigation of neuronal organisation because the motor pattern is expressed in a stereotyped manner and the nervous system is readily accessible to electrophysiological recording techniques. The mechanical properties of the wings and their role in sound generation have been described (Koch et al. 1988), and the activity of specific muscles has been correlated with wing movement during sound production (Bentley and Kutsch 1966, Kutsch 1969, Bentley 1971). Sensory feedback from wing hinge and wing surface receptors is important for efficient sound production (Elliott 1983, Elliott and Koch 1983, Schöffner and Koch 1987), although pattern generation itself appears to be independent of this feedback (Kutsch and Huber 1970, Möss 1971, Schöffner and Koch 1987). Electrical stimulation of selected parts of the central nervous system such as the brain (Huber 1960a), the cervical connectives (Otto 1971) and presumed single fibres within these connectives (Bentley 1977) showed that the neural circuitry generating the song pattern is located in the thoracic nervous system (Kutsch and Otto 1972), whereas the brain orchestrates the different song patterns (Huber 1960a, Otto 1971, Bentley 1977). Bentley (1969a) has described the activity of motoneurons and unidentified interneurons within the meso- and metathoracic ganglia during stridulation in *Gryllus campestris*.

Different species of crickets have evolved different calling songs in order to attract conspecific females. The muscular and motoneuronal activity recorded during cricket stridulation can help to resolve questions relevant to the understanding of motor pattern generation such as: what are the properties of the song pattern generator responsible for the species-specific differences in the song? Further, some species of the same genus (*Teleogryllus*) produce calling songs which contain two different temporal rhythms (Bentley 1971). Are two pattern generators then used during the expression of the one behaviour?

The first step necessary for answering these questions involves a description of the activity of muscles and motoneurons during stridulation. The cricket *Teleogryllus commodus* was chosen for two reasons: firstly, the *T.commodus* calling song contains two rhythms: the chirp and the trill. During the latter the syllables are produced at double the repetition rate of those in the chirp (Bentley 1971). Secondly, most studies on muscular and motoneuronal activity during cricket stridulation have focussed on *G.campestris*, which has a distinctly different stridulatory pattern from *T.commodus*. Thus a comparison between the neuronal

control of the calling songs of the two species might reveal common principles in the neuromuscular control of the forewings during cricket stridulation.

In order to induce stridulation routinely in intact as well as in dissected animals, a method recently developed to elicit stridulation in grasshoppers (Hedwig 1986) was modified for the cricket. This technique involved applying DC-current to the brain and proved to be superior in reliability to the focal lesions and pulsed trains of electrical stimuli used previously (Huber 1960a, Bentley 1969a, Otto 1971, Bentley 1977). In this paper the discharge pattern of opener and closer muscles during stridulation in *T.commodus* is first described quantitatively, and this is followed by a description of the intracellular activity of some of the motoneurons active during stridulation. Motoneurons innervating opener muscle 99 (subalar) and closer muscle 90 (remotor coxae) were chosen for investigation because their activity is readily recorded and has been used previously to describe the stridulatory motor pattern in *G.campestris*. The investigation of synaptic input to closer and opener motoneurons in the present study indicates that some premotor interneurons are active during both the chirp and the trill, whereas others are active only during the chirp.

3. MATERIALS AND METHODS

3.1. Animals

Experiments were performed on adult male crickets, *Teleogryllus commodus* (Walker), from 1 to 5 weeks post-ecdysis. Crickets were either caught in the field near Canberra, Australia, or taken from a breeding colony not more than two generations old, originating from the same area.

3.2. Eliciting stridulation

All legs were removed and the crickets were restrained, dorsal side up, by pinning the head capsule onto a plasticine cushion supported by foam. The anterior head capsule, including the antennae, the clypeus, and the labrum, was removed to expose the brain (Fig. 2.1 B, C). These were the only dissections on the otherwise intact animal. A suction electrode, consisting of a plastic tube mounted onto the front of a shortened syringe needle and filled with saline, was applied to the ventral part of the brain, just anterior to the

circumoesophageal connectives (Fig. 2.1 B). The inner diameter of the suction electrode was approximately 0.5 mm. Electrodes of smaller diameter elicited stridulation less readily. The indifferent electrode was placed approximately 0.5 mm posterior to the suction electrode.

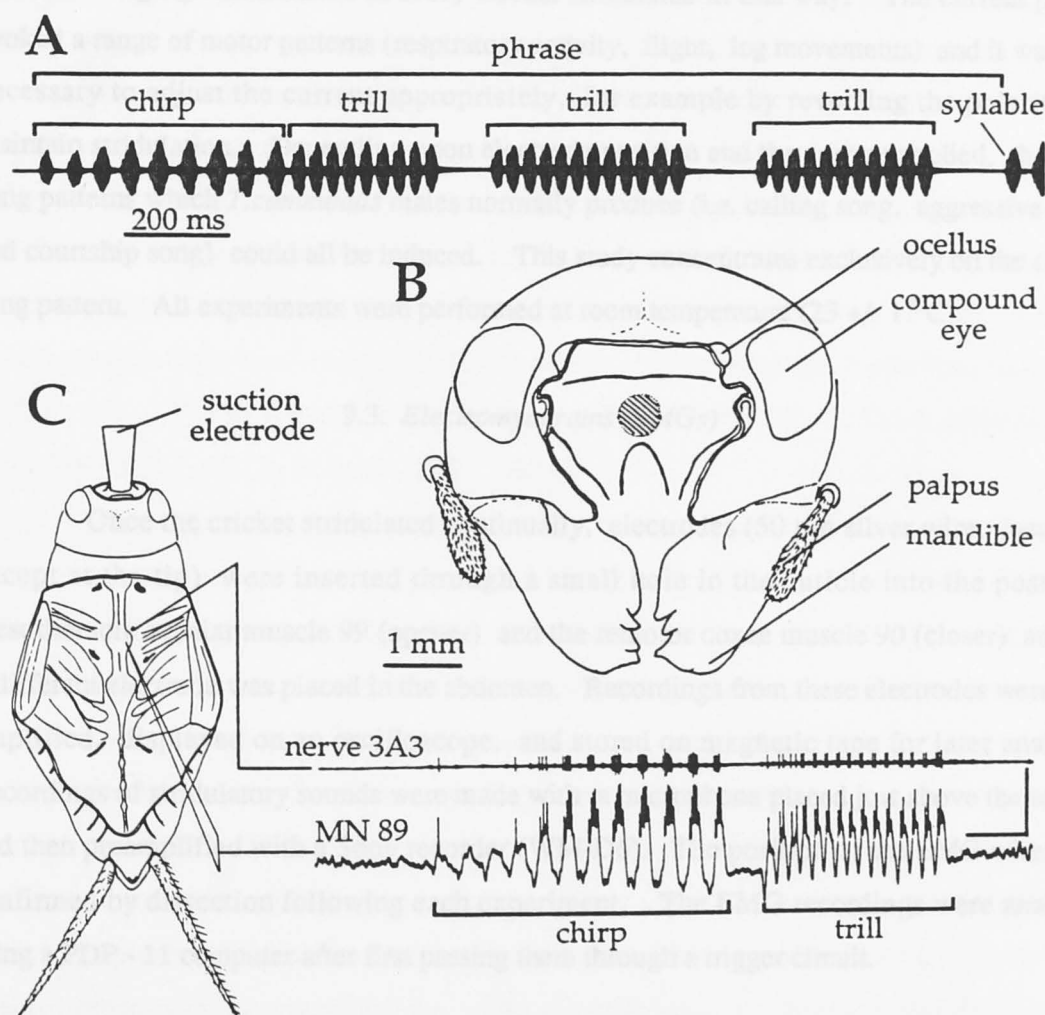


Fig. 2.1 A - C. *Teleogryllus commodus* calling song structure and the experimental arrangement for electrophysiological stimulation and recording. A Calling song of an unrestrained, voluntarily singing cricket. The two different rhythms (chirp, trill) are indicated and together they form a phrase which is continuously repeated. B The ventral surface of the brain as seen from the animal's anterior. The placement and the size of the inner diameter of the stimulating suction electrode are indicated by the shaded area. C Stridulation in intact (not shown) and dissected cricket males was induced by passing a negative DC current ($\sim 20 - 40 \mu\text{A}$) and intracellular recordings from motoneurons during stridulation were made from the mesothoracic ganglion. A hook electrode placed under nerve 3A3, which innervates closer muscle 89, was used as a monitor for ongoing stridulation. The motoneuronal recording, from a motoneuron 89, shows an electrically induced chirp/trill pattern. Calibration: Vertical 20 mV; Horizontal 100 ms.

To elicit stridulation a negative DC current of 20 - 40 μ A (generated from an 18 V battery) was applied to the brain. As soon as current was applied the cricket showed an increase in ventilatory and other motor activity and often struggled. Within 1 - 5 minutes the cricket usually began to stridulate, sometimes continually for up to 3 hrs. At least a short period of singing was induced in every cricket stimulated in this way. The current passed evoked a range of motor patterns (respiratory activity, flight, leg movements) and it was then necessary to adjust the current appropriately, for example by reversing the polarity, to maintain stridulation. Depending upon electrode position and the current applied, the three song patterns which *T.commodus* males normally produce (i.e. calling song, aggressive song, and courtship song) could all be induced. This study concentrates exclusively on the calling song pattern. All experiments were performed at room temperature (23 \pm 1 $^{\circ}$ C).

3.3. Electromyograms (EMGs)

Once the cricket stridulated continually, electrodes (50 μ m silver wire, insulated except at the tip) were inserted through a small hole in the cuticle into the posterior mesothoracic subalar muscle 99 (opener) and the remotor coxae muscle 90 (closer) and the indifferent electrode was placed in the abdomen. Recordings from these electrodes were then amplified, displayed on an oscilloscope, and stored on magnetic tape for later analysis. Recordings of stridulatory sounds were made with a microphone placed just above the animal and then preamplified with a Sony recorder (WM-D6). The position of the EMG wires was confirmed by dissection following each experiment. The EMG recordings were analysed using a PDP - 11 computer after first passing them through a trigger circuit.

3.4. Preparation for intracellular recordings

Stridulation was induced as described above and maintained throughout the dissection procedure. The wings and the posterior part of the pronotum were removed, and a dorsal midline incision allowed the meso- and metathoracic ganglia to be exposed following removal of the gut and overlying tissue (Fig. 2.1 C; see also Robertson 1987). These ganglia were then supported on a Nichrome platform and particular care was taken not to damage or stretch the abdominal connectives, since eliciting stridulation then proved much more difficult and often impossible. All sensory and motor nerves to the thoracic ganglia were cut so as to

reduce body movements and obtain a preparation in which the thoracic nervous system generating the motor pattern was as completely deafferented as possible. This operation had some effect on the EMG and central neuronal recordings, most likely due to removal of sensory feedback from proprioceptors in the wingstumps and on the wing surface. Reafferent activity from the cerci (Dambach et al. 1983) was probably also lacking under these circumstances, because no wing movements or muscular contractions were present.

To monitor ongoing stridulation, usually nerve 3A3 of the mesothoracic ganglion (which contains motor axons innervating the anterior closer muscle 89 (Fig. 2.1 C)) was lifted onto a 75 μm silver wire hook electrode and insulated from body fluids with a mixture of vaseline and mineral oil. Sometimes nerve 3A was used as a monitor, in which case recordings then contained background activity of motoneurons innervating muscles other than muscle 89 (see Kutsch and Huber (1970) for details of innervation and the nomenclature used in this study). The preparation was continuously flooded with TES-buffered saline (in mM: NaCl 128, KCl 1, CaCl_2 2, NaHCO_3 2, TES 4).

Glass capillary micropipettes pulled to a resistance of 40 - 60 M Ω (when filled with 1 M potassium acetate) were backfilled with a 3% solution of Lucifer Yellow in 0.5 M lithium chloride for intracellular staining. Motoneurons and interneurons were penetrated in their neuropilar segments and intracellular activity was recorded using standard techniques and stored on magnetic tape. Recorded neurons were then stained by applying constant hyperpolarising current (5 - 10 nA) for 5 - 15 min. The ganglion was dissected from the preparation, fixed in 3% phosphate buffered (pH=7.2) formaldehyde, dehydrated, cleared, and viewed and drawn as a wholemount under a fluorescence microscope. The intracellular recordings presented in this study are representative of those obtained for each motoneuron type in more than 20 preparations. The nomenclature of interneurons follows the classification scheme devised by Robertson and Pearson (1983) and Robertson (1987) and is based on soma position in the ganglion and axon path in the connective. Each interneuron's identification number is prefixed with the letter *T* (for *Teleogryllus*) as previously done by Robertson (1987) to distinguish these interneurons from interneurons with the same identification number which have previously been identified in the locust.

4. RESULTS

4.1. *Eliciting stridulation with electrical DC-stimulation of the brain*

Stridulation in *T.commodus* consists of a repetition of phrases (Fig. 2.1 A), each phrase usually commencing with a short chirp (4-6 syllables at ~ 20 Hz) and ending with one to three trills (10 to 30 syllables at ~ 35 Hz). Stridulation was most successfully induced by locating the suction electrode on the ventral side of the brain just anterior to the circumoesophageal connectives (Fig. 2.1 B), and then applying constant, negative DC-current (Fig. 2.1 C). The stridulatory behaviour evoked appeared normal in that the forewings were raised to the normal angle, and each syllable in the typical calling song was produced by closing movements of the forewings. The stridulatory pattern evoked by electrical stimulation clearly shows the same phrase structure as above, but the chirp was often extended (> 10 syllables) and the trill drastically shortened (Fig. 2.2 A). Male crickets produce such a song pattern normally during a warm-up period before a continuous song can be observed. Sometimes the stridulation elicited was indistinguishable from normal stridulation in having short chirps and long trills. There appeared to be no significant difference between the period of sound pulses in normal and electrically induced stridulation (chirp: 51.1 +/- 3.3 ms and 51.8 +/- 4.3 ms respectively; trill: 31.4 +/- 1.1 ms and 29.9 +/- 1.2 ms respectively; means and standard deviations are from five animals in each group).

4.2. *Neuromuscular activity during stridulation*

EMG recordings from intact singing crickets were made from muscle 99 (opener) and muscle 90 (closer), together with sound recordings. The increasing amplitude of sound pulses reflects the increase in excitation seen in closer muscle bursts during the chirp (Fig. 2.2 A). In order to characterise the pattern of activity in these muscles during stridulation the intervals between the opener burst and the closer burst and vice versa were measured as indicated in Fig. 2.2 B. The opener to closer interval is always the shorter interval and thus the period is unevenly divided. Both intervals vary monotonically with period duration (Fig. 2.2 C) but occur at approximately constant phases within the stridulatory cycle (opener to closer: 0.37 +/- 0.06; closer to opener: 0.63 +/- 0.08). A phrase always starts with an opener burst and ends with a closer burst (Fig. 2.2 A). Measuring the burst durations

reveals that the opener burst is very short (often consisting of only a single spike) in both trill and chirp and is independent of period (Fig. 2.2 A, D). By contrast the closer burst duration in the chirp increases, particularly with longer periods (Fig. 2.2 B, D). This increase in burst duration during the chirp is similar to the one observed for the closer to opener interval (Fig. 2.2 C), which results in an approximately constant interval from the end of the closer burst to the beginning of the opener burst (18.7 ± 3.9 ms).

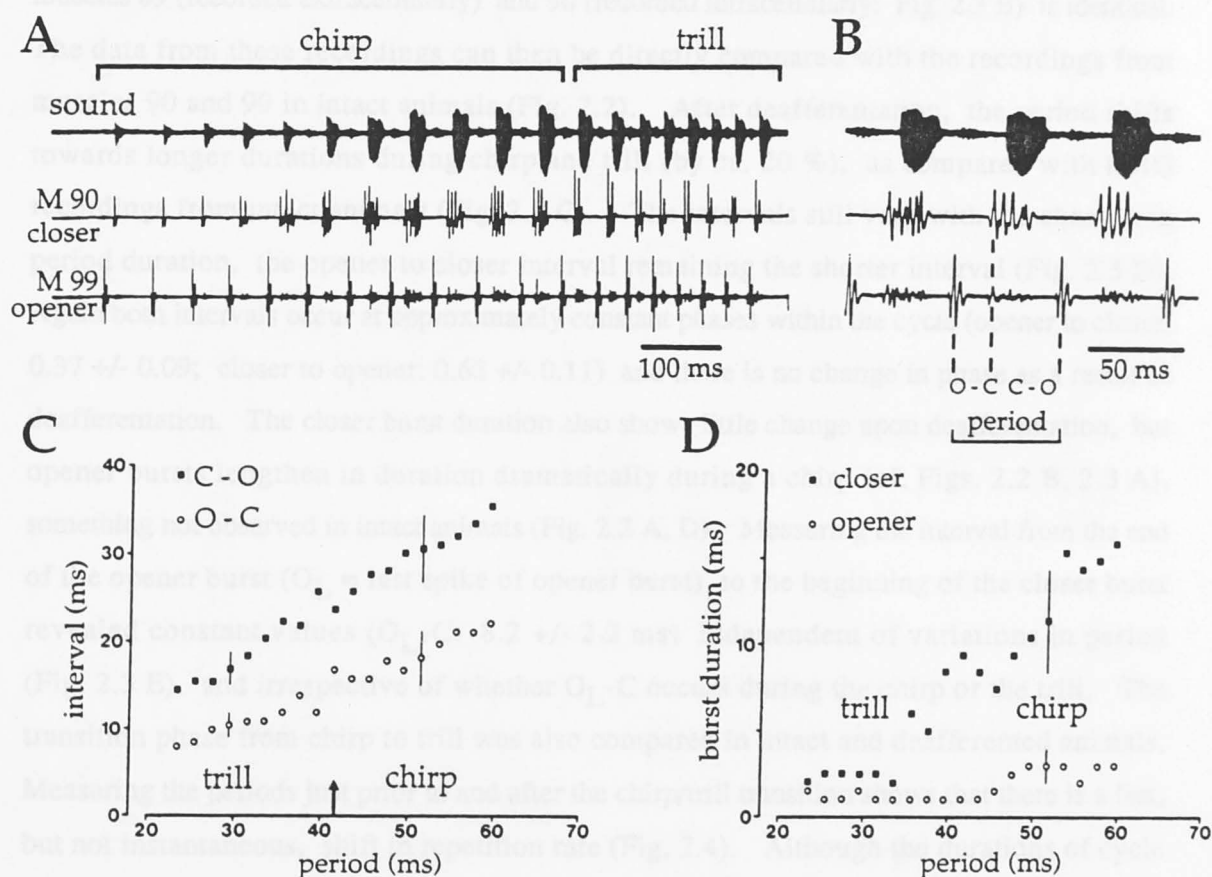


Fig. 2.2 A - D. Electromyogram analysis of the stridulatory pattern produced by an intact singing cricket male. **A** Sound pattern of the *T.commodus* calling song and the underlying activity of closer muscle 90 (upper trace) and opener muscle 99 (lower trace) induced by electrical stimulation. Only the closing wing stroke produces a sound pulse. Stridulation begins with an opener burst and ends with a closer burst. **B** Indication of the parameters analysed from EMG recordings. Period is measured from opener burst to opener burst. The intervals were measured from the beginning of a burst to the beginning of the antagonistic burst. O: Opener, C: Closer. **C** Both intervals (O-C, C-O) vary with changing period durations. Arrow indicates period durations occurring at the transition from chirp to trill. **D** Opener burst duration changes only little with varying periods, whereas closer burst duration increases during the chirp.

Dissection of the cricket for intracellular analysis resulted in removal of peripheral feedback from thoracic proprioceptors and the cerci to the pattern generator. In order to assess the impact of this deafferentation on the motor output, the period, interval, and burst durations of activity in opener and closer muscles were examined. Intracellular recordings of opener motoneuron 99 were compared with extracellular recordings from the motoneurons innervating closer muscle 89 (Fig. 2.3 A), because the timing of excitatory input to closer muscles 89 (recorded extracellularly) and 90 (recorded intracellularly; Fig. 2.3 B) is identical. The data from these recordings can then be directly compared with the recordings from muscles 90 and 99 in intact animals (Fig. 2.2). After deafferentation, the period shifts towards longer durations during chirp and trill (by ca. 20 %), as compared with EMG recordings from intact animals (Fig. 2.3 C). The intervals still vary with the changes in period duration, the opener to closer interval remaining the shorter interval (Fig. 2.3 D). Again both intervals occur at approximately constant phases within the cycle (opener to closer: 0.37 ± 0.09 ; closer to opener: 0.63 ± 0.11) and there is no change in phase as a result of deafferentation. The closer burst duration also shows little change upon deafferentation, but opener bursts lengthen in duration dramatically during a chirp (cf. Figs. 2.2 B, 2.3 A), something not observed in intact animals (Fig. 2.2 A, D). Measuring the interval from the end of the opener burst (O_L = last spike of opener burst) to the beginning of the closer burst revealed constant values (O_L -C: 8.2 ± 2.2 ms) independent of variations in period (Fig. 2.3 E), and irrespective of whether O_L -C occurs during the chirp or the trill. The transition phase from chirp to trill was also compared in intact and deafferented animals. Measuring the periods just prior to and after the chirp/trill transition shows that there is a fast, but not instantaneous, shift in repetition rate (Fig. 2.4). Although the durations of cycle periods in intact and deafferented animals differ (Fig. 2.3 C) the pattern of change occurring at the chirp/trill transition is very similar in both conditions (Fig. 2.4). Extrapolating the chirp period revealed no apparent phase preference for the commencement of trill bursts in either intact or deafferented animals (not shown).

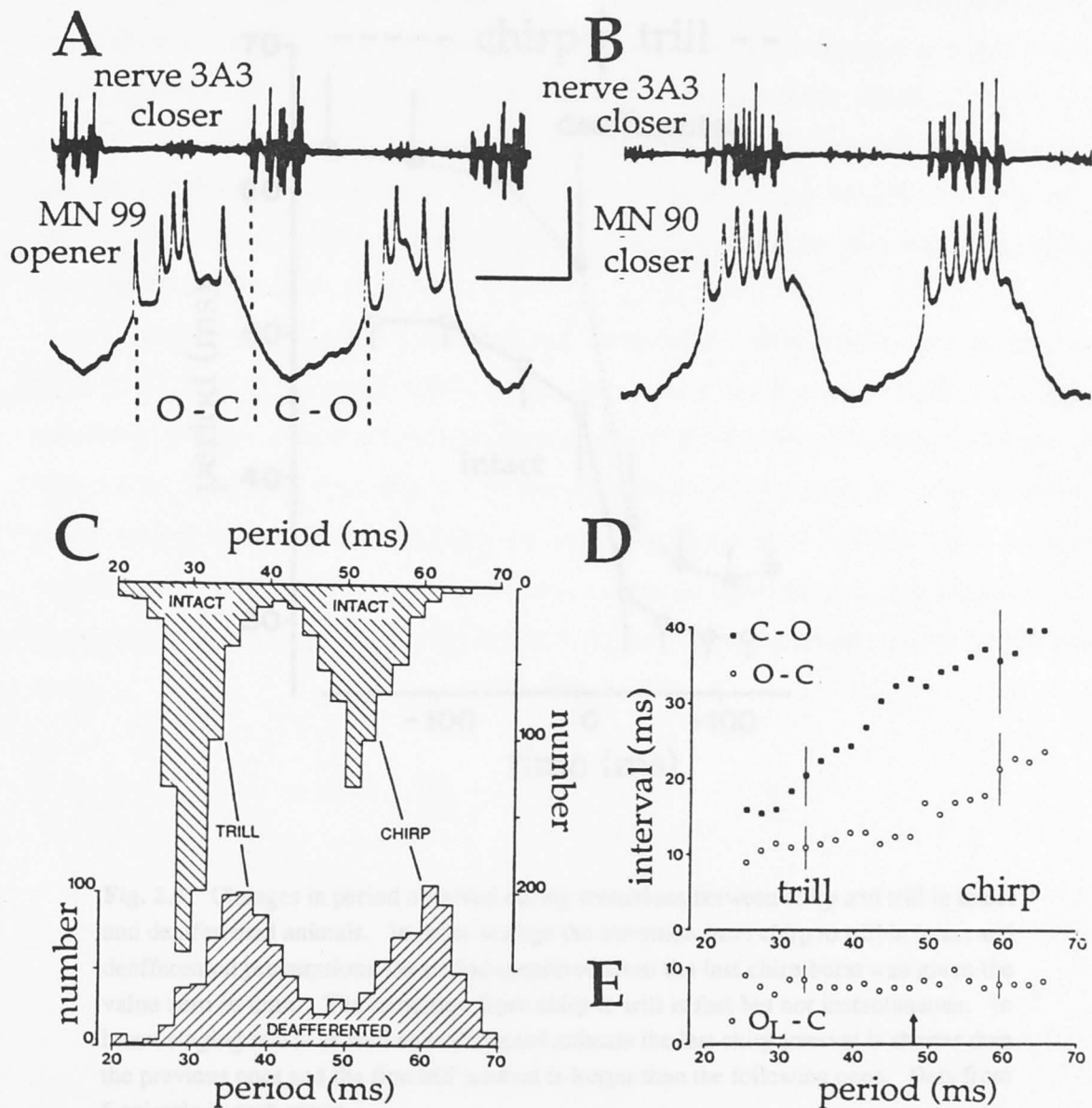


Fig. 2.3 A - E Stridulation after deafferentation of the thoracic nerve cord. **A** Measurements were taken from recordings from nerve 3A3 which innervates closer muscle 89 and intracellular recordings from opener MN 99. **B** Closer MN 90 and closer muscle 89 are activated simultaneously thus allowing a comparison with data from intact animals (see Fig. 2.2). Calibration for **A**, **B**: Vertical 15 mV, Horizontal 25 ms. **C** Deafferentation causes an increase in period duration by ca. 20 % (upper histogram: intact; lower histogram: deafferented). **D** Both intervals vary with period. **E** Measuring from the last spike of the opener burst to the beginning of the closer burst (O_L -C) reveals intervals of constant value irrespective of period. Arrow indicates period durations occurring at the transition from chirp to trill.

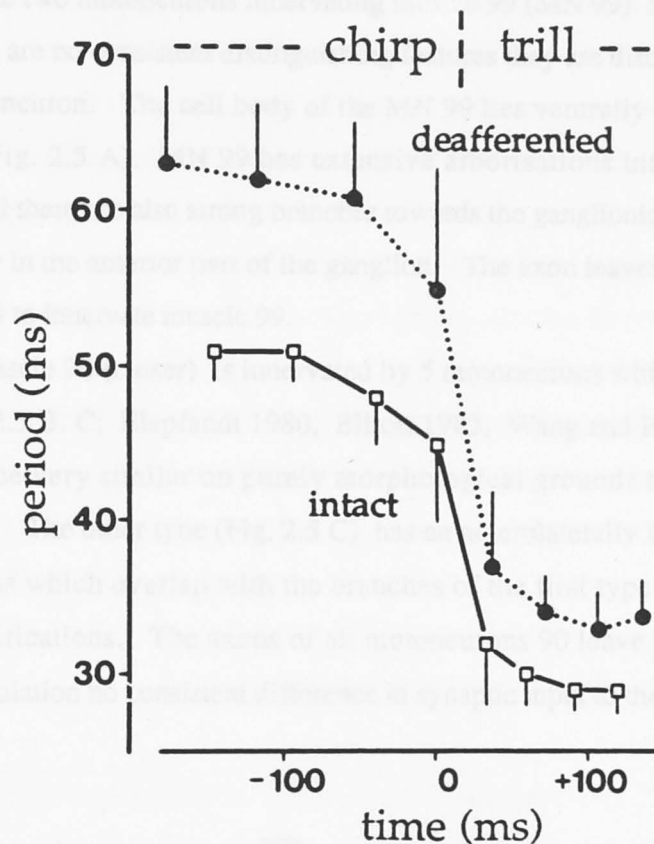


Fig. 2.4 Changes in period observed during transitions between chirp and trill in intact and deafferented animals. In order to align the transition from chirp to trill in intact and deafferented preparations the period measured from the last chirp burst was given the value zero in time. The transition from chirp to trill is fast but not instantaneous. In intact singing males as well as in dissected animals the last chirp interval is shorter than the previous ones and the first trill interval is longer than the following ones. Data from 5 animals in each group.

4.3. Structure of motoneurons

Only the pattern of synaptic input to motoneurons innervating the opener muscle 99 and its direct antagonist, closer muscle 90, has been investigated in this study. The structure of these motoneurons has been described previously for *G.campestris* (Elepfandt 1980, Elliott 1983) and *Teleogryllus oceanicus* (Wang and Robertson 1989), and as their structures are very similar, will only briefly be outlined for *T.commodus* (Fig. 2.5).

The two motoneurons innervating muscle 99 (MN 99) are very similar in structure, and as there are no consistent distinguishing features they are discussed here as if they were a single motoneuron. The cell body of the MN 99 lies ventrally at the posterior edge of the ganglion (Fig. 2.5 A). MN 99 has extensive arborisations into the posterior half of the ganglion and there are also strong branches towards the ganglionic midline as well as laterally and dorsally in the anterior part of the ganglion. The axon leaves the mesothoracic ganglion via nerve 4D to innervate muscle 99.

Muscle 90 (closer) is innervated by 5 motoneurons which can be grouped into two types (Fig. 2.5 B, C; Elepfandt 1980, Elliott 1983, Wang and Robertson 1989). One type appears to be very similar on purely morphological grounds to MN 99 described above (Fig. 2.5 B). The other type (Fig. 2.5 C) has an anterolaterally located cell body and dorsal arborisations which overlap with the branches of the first type of MN 90. There are also ventral ramifications. The axons of all motoneurons 90 leave the ganglion via nerve 4D. During stridulation no consistent difference in synaptic input to these two types of MN 90 was noted.

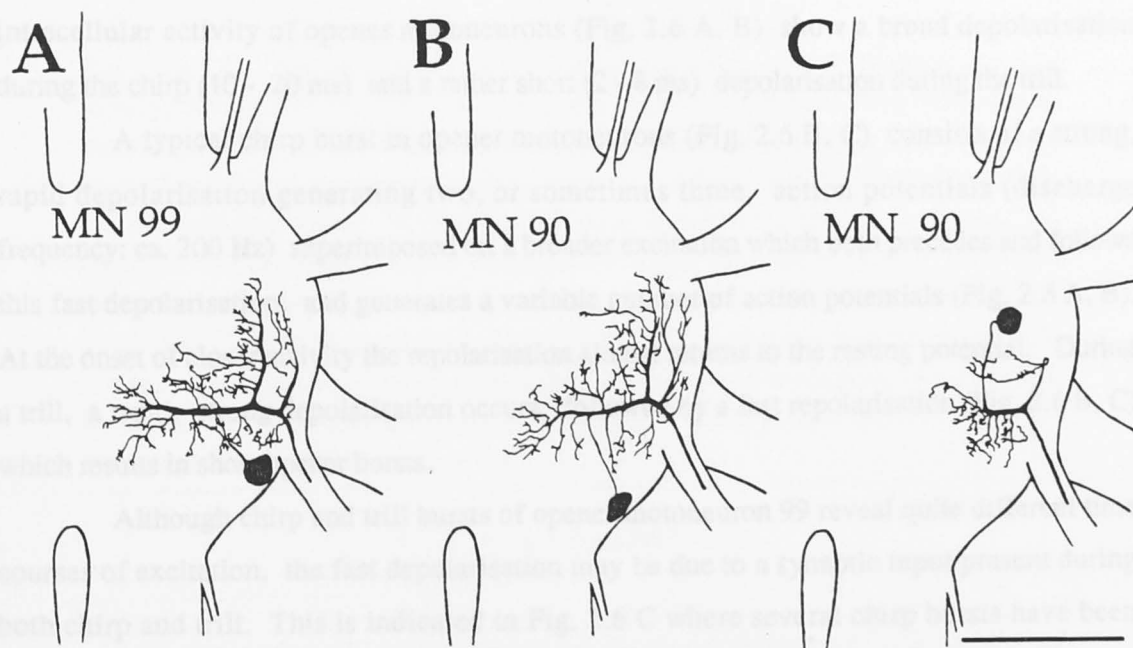


Fig. 2.5 A - C. Drawings showing the morphology of mesothoracic MNs 90 and 99 as seen in wholemount following intracellular staining with Lucifer Yellow. **A** Subalar muscle 99 is innervated by two MNs, which appeared morphologically indistinguishable. Nerve roots N 3, N 4, and N 5 are indicated. **B, C** Remotor coxae muscle 90 is innervated by five MNs, four with a posterior (**B**), one with a more anterior cell body (**C**). Scale: 200 μ m.

4.4. Patterns of synaptic input to motoneurons during stridulation

To record the activity of identified motoneurons 99 (opener) and 90 (closer), stridulation was first induced electrically in an *intact* animal. Once stridulation continued regularly the animal was dissected as described above (Fig. 2.1 C). The intracellularly recorded activity in motoneurons reflects the chirp/trill sequences typical of electrically induced singing (Fig. 2.1 C, 2.2 A). A continuous stridulatory motor pattern with brief chirps and long trills occurred only rarely.

The pattern of synaptic input to opener and closer motoneurons was examined to determine whether motoneurons receive different input during the chirp and trill, or whether the pattern of synaptic input during a chirp merely represents a slowed version of that occurring during the trill.

4.4.1. Opener motoneurons

The opening movement of the wings is soundless (Fig. 2.2 A, B). The recordings of intracellular activity of opener motoneurons (Fig. 2.6 A, B) show a broad depolarisation during the chirp (10 - 20 ms) and a rather short (2 - 8 ms) depolarisation during the trill.

A typical chirp burst in opener motoneurons (Fig. 2.6 B, C) consists of a strong, rapid depolarisation generating two, or sometimes three, action potentials (discharge frequency: ca. 200 Hz) superimposed on a broader excitation which both precedes and follows this fast depolarisation, and generates a variable number of action potentials (Fig. 2.6 A, B). At the onset of closer activity the repolarisation almost returns to the resting potential. During a trill, a rapid, strong depolarisation occurs, followed by a fast repolarisation (Fig. 2.6 B, C) which results in short opener bursts.

Although chirp and trill bursts of opener motoneuron 99 reveal quite different time courses of excitation, the fast depolarisation may be due to a synaptic input present during both chirp and trill. This is indicated in Fig. 2.6 C where several chirp bursts have been aligned above a trill burst. The chirp bursts just prior to the chirp/trill transition also show a decrease in the broad excitation leaving only the central depolarisation to form the trill burst (Fig. 2.6 B).

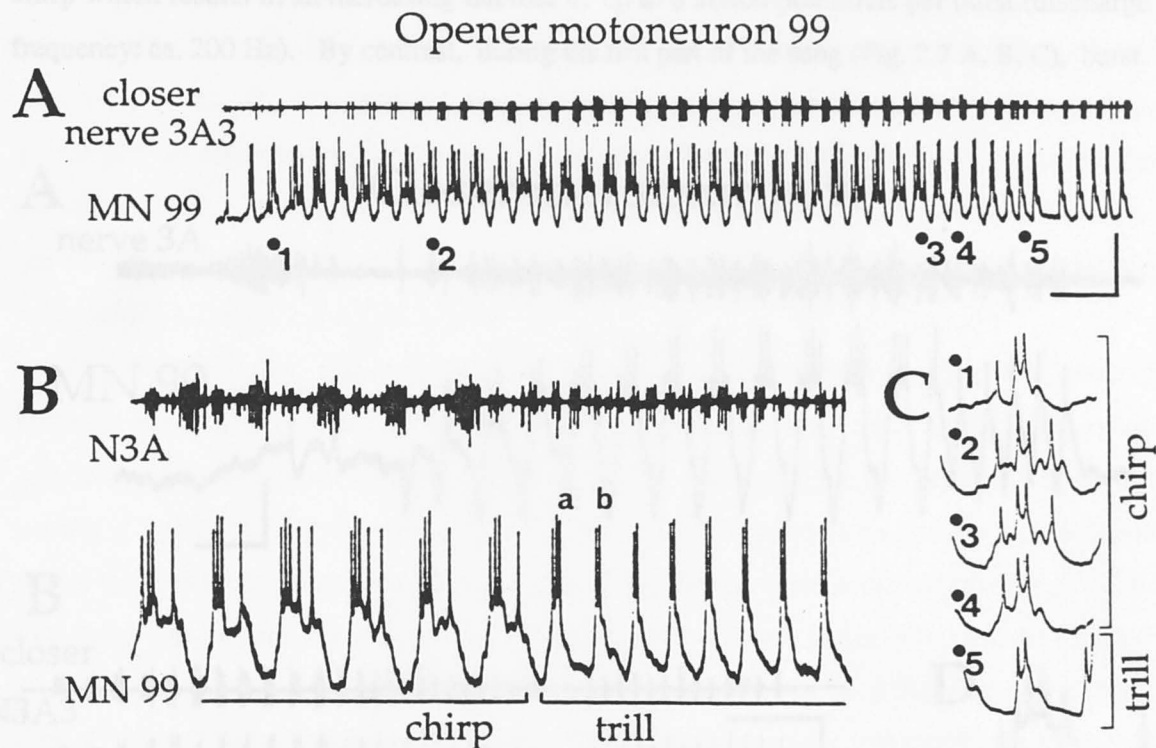


Fig. 2.6 A - C. Intracellular recordings from opener motoneuron 99 during electrically induced stridulatory activity. **A** Complete stridulatory phrase. The extracellular recording of nerve 3A3 monitors activity of closer muscle 89 (see Fig. 2.1 C). The chirp shows a typical fast depolarisation which supports a variable number of action potentials. **B** Expanded section of a chirp/trill transition in a different preparation. During the transition the late depolarisation disappears first (see first trill burst (a)), before the excitation generating the first action potential in this example disappears (see second trill burst (b)). **C** Aligning several chirp bursts above a trill burst taken from the recording in **A** (the numbers indicate the respective bursts) shows the additional synaptic input which distinguishes chirp from trill bursts. Calibration: Vertical **A**, **C** 20 mV **B** 10 mV; Horizontal **A** 200 ms **B** 65 ms **C** 40 ms.

4.4.2. Closer motoneurons

Only the closing wing stroke generates the sound pulses in an intact animal (Fig. 2.2 A, B). During the chirp the syllables initially show a progressive increase in amplitude and duration (Fig. 2.2 A). Intracellular recordings made during a chirp (Fig. 2.7 A, B) reveal inhibitory and excitatory input to closer motoneurons. The synaptic input to MN 90 during a chirp (Fig. 2.7 A, C) consists of repeated cycles in each of which an IPSP is followed by a strong excitation. The excitatory input shows a progressive increase in amplitude through a

chirp which results in an increasing number of up to 6 action potentials per burst (discharge frequency: ca. 200 Hz). By contrast, during the trill part of the song (Fig. 2.7 A, B, C), burst

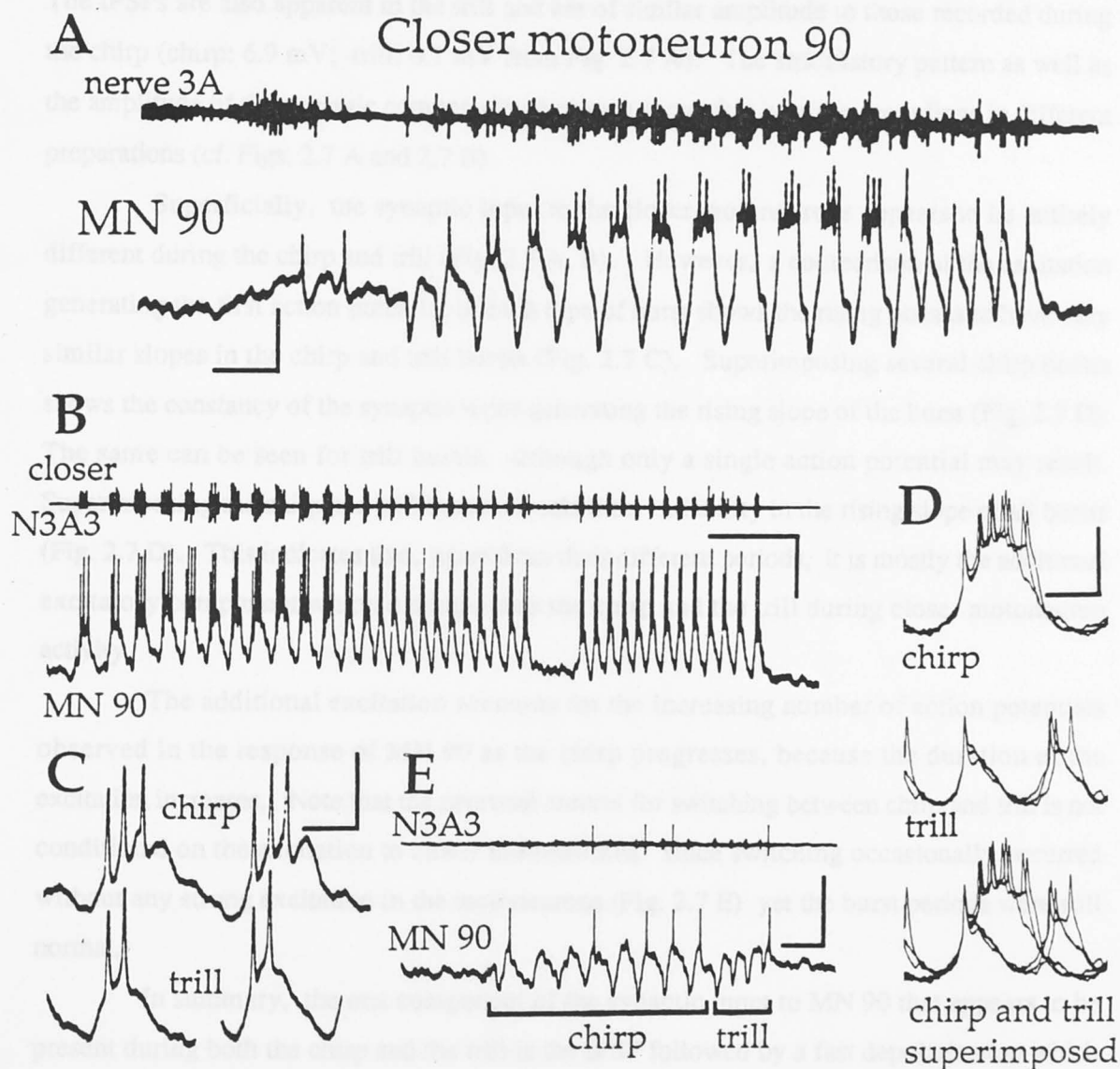


Fig. 2.7 A - E. Activity of closer motoneuron 90 recorded intracellularly during electrically induced stridulatory activity. A, B Sequence of a complete stridulatory phrase. The extracellular recording of nerve 3A monitors closer activity of muscle 89 (see Fig. 2.1 C). Note the variation in stridulatory pattern and amplitude of synaptic events between these two examples. C Enlarged chirp and trill bursts illustrate the synaptic input (from B). D Superimposing chirp and trill sections illustrates the common pattern of synaptic input to this MN during chirp and trill. E Excitation in closer MNs is not necessary for switching from chirp to trill. Calibration: Vertical A, B, C 10 mV D, E 15 mV; Horizontal A, E 100 ms B 200 ms C 20 ms D 25 ms.

durations are shorter and show a weaker excitation following the initial fast depolarisation (amplitude: chirp: 22 mV; trill: 16 mV; duration: chirp: 30 ms; trill: 16 ms in the example shown in Fig. 2.6 D). This corresponds to the lower intensity syllables generated during a trill. The IPSPs are also apparent in the trill and are of similar amplitude to those recorded during the chirp (chirp: 6.9 mV; trill: 6.1 mV from Fig. 2.7 A). The stridulatory pattern as well as the amplitude of the synaptic components may vary somewhat between recordings in different preparations (cf. Figs. 2.7 A and 2.7 B).

Superficially, the synaptic input to the closer motoneurons appears to be entirely different during the chirp and trill (Fig. 2.7 A, B). However, a comparison of the excitation generating the first action potential in each type of burst shows the rising phases to have very similar slopes in the chirp and trill bursts (Fig. 2.7 C). Superimposing several chirp bursts shows the constancy of the synaptic input generating the rising slope of the burst (Fig. 2.7 D). The same can be seen for trill bursts, although only a single action potential may result. Superimposing the chirp and trill bursts reveals little variability in the rising slope of all bursts (Fig. 2.7 D). This indicates that, apart from their different periods, it is mostly the additional excitatory component which distinguishes the chirp and the trill during closer motoneuron activity.

The additional excitation accounts for the increasing number of action potentials observed in the response of MN 90 as the chirp progresses, because the duration of the excitation increases. Note that the neuronal control for switching between chirp and trill is not conditional on the excitation to closer motoneurons, since switching occasionally occurred without any strong excitation in the motoneurons (Fig. 2.7 E) yet the burst periods were still normal.

In summary, the one component of the synaptic input to MN 90 that appears to be present during both the chirp and the trill is the IPSP followed by a fast depolarisation which then generates one action potential. This initial depolarisation is quickly followed by an even stronger excitation which is expressed during the chirp but not during the trill (Fig. 2.7 D).

4.5. Interneuronal burst patterns

In an attempt to obtain some insight as to how the motoneuronal burst patterns are generated and how the switching from chirp to trill is organised by the nervous system, seven interneurons rhythmically active with the stridulatory rhythm were identified (Fig. 2.8). All

stridulatory interneurons

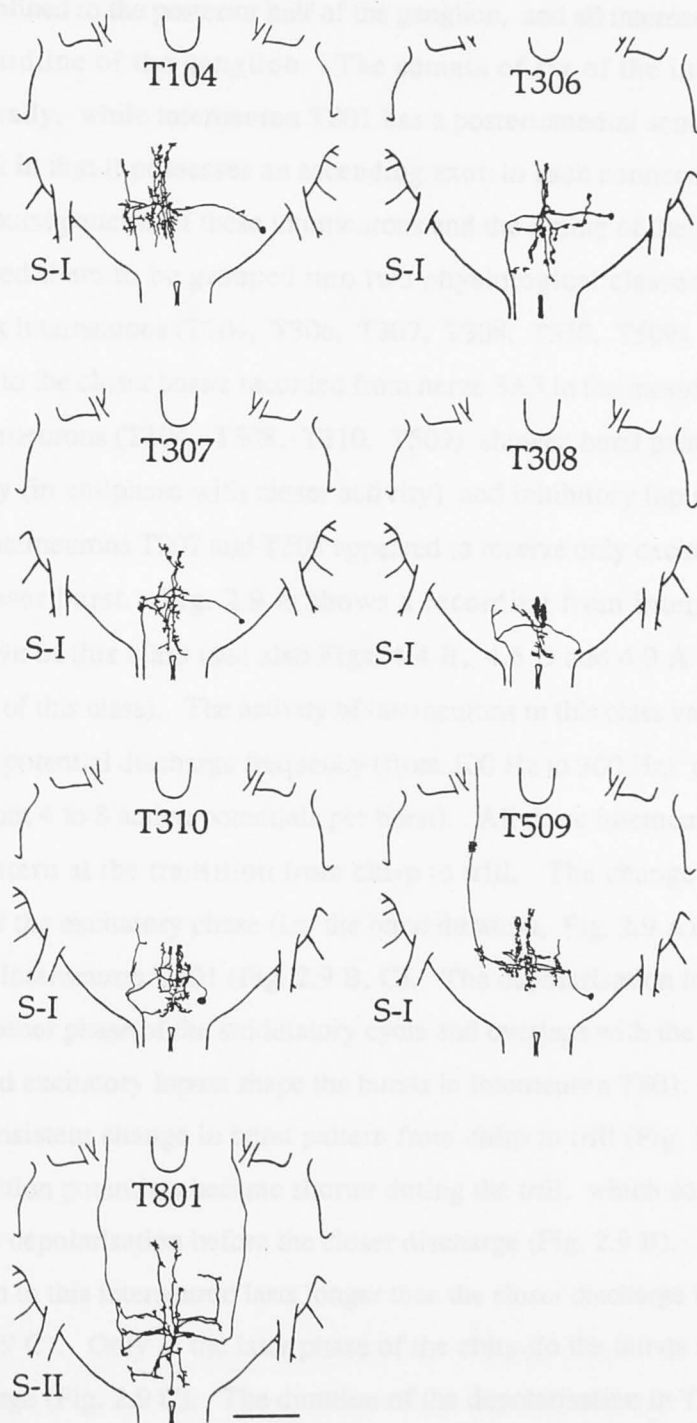


Fig. 2.8. Drawings showing the morphology of metathoracic stridulatory interneurons as seen in wholemount following intracellular staining with Lucifer Yellow. Interneurons T104, T306, T307, T308, T310, T509, T801. The Roman numerals refer to the classes S-I and S-II discussed in the text (see also Fig. 2.9). Four interneurons show an axon descending to the abdominal nerve cord, two have ascending axons and one is a local interneuron. Scale 200 μ m.

interneurons reported here are located in the metathoracic ganglion. Their major branching areas are confined to the posterior half of the ganglion, and all interneurons show arborisations along the midline of the ganglion. The somata of six of the interneurons are located posteriolaterally, while interneuron T801 has a posteromedial soma. Interneuron T801 is also unusual in that it possesses an ascending axon in each connective of the ventral nerve cord. The burst patterns of these interneurons and the timing of their bursts in a stridulatory cycle allowed them to be grouped into two physiological classes (Fig. 2.9). *Class S-I* contained six interneurons (T104, T306, T307, T308, T310, T509) which were depolarised in antiphase to the closer bursts recorded from nerve 3A3 in the mesothoracic ganglion. Four of these interneurons (T104, T308, T310, T509) showed burst patterns which were shaped by excitatory (in antiphase with closer activity) and inhibitory inputs (at the time of closer activity). Interneurons T307 and T308 appeared to receive only excitatory inputs in antiphase with the closer burst. Fig. 2.9 A shows a recording from interneuron T308 which is representative of this class (see also Figs. 4.4 B, 4.6 D and 4.9 A for other recordings of interneurons of this class). The activity of interneurons in this class varied mainly with respect to the action potential discharge frequency (from 100 Hz to 300 Hz) and the action potentials per burst (from 4 to 8 action potentials per burst). All these interneurons showed changes in the burst pattern at the transition from chirp to trill. The change usually consisted of a shortening of the excitatory phase (i.e. the burst duration, Fig. 2.9 A). *Class S-II* consists to date only of interneuron T801 (Fig. 2.9 B, C). The depolarisation in this interneuron starts during the opener phase of the stridulatory cycle and overlaps with the closer discharge. Both inhibitory and excitatory inputs shape the bursts in interneuron T801. Interneuron T801 also showed a consistent change in burst pattern from chirp to trill (Fig. 2.9 B). The excitation generating action potentials became shorter during the trill, which sometimes resulted in the ending of the depolarisation before the closer discharge (Fig. 2.9 B). On the other hand, the depolarisation in this interneuron lasts longer than the closer discharge in the early phase of the chirp (Fig. 2.9 C). Only in the later phase of the chirp do the bursts in T801 end before the closer discharge (Fig. 2.9 C). The duration of the depolarisation in T801 changes only very little as the chirp progresses while the amplitude of the excitatory input increases. This increase is paralleled in the closer discharge which increases in duration, although the cycle period remains almost constant (Fig. 2.9 C). Representatives of other possible classes of stridulatory interneurons were not encountered during this investigation (see, however, Chapter 3 for such interneurons active during the expression of the flight rhythm).

Experimental current injection during stridulation was attempted, but none of the 7 interneurons (Fig. 2.8) described here could be shown unequivocally to change the stridulatory burst pattern.

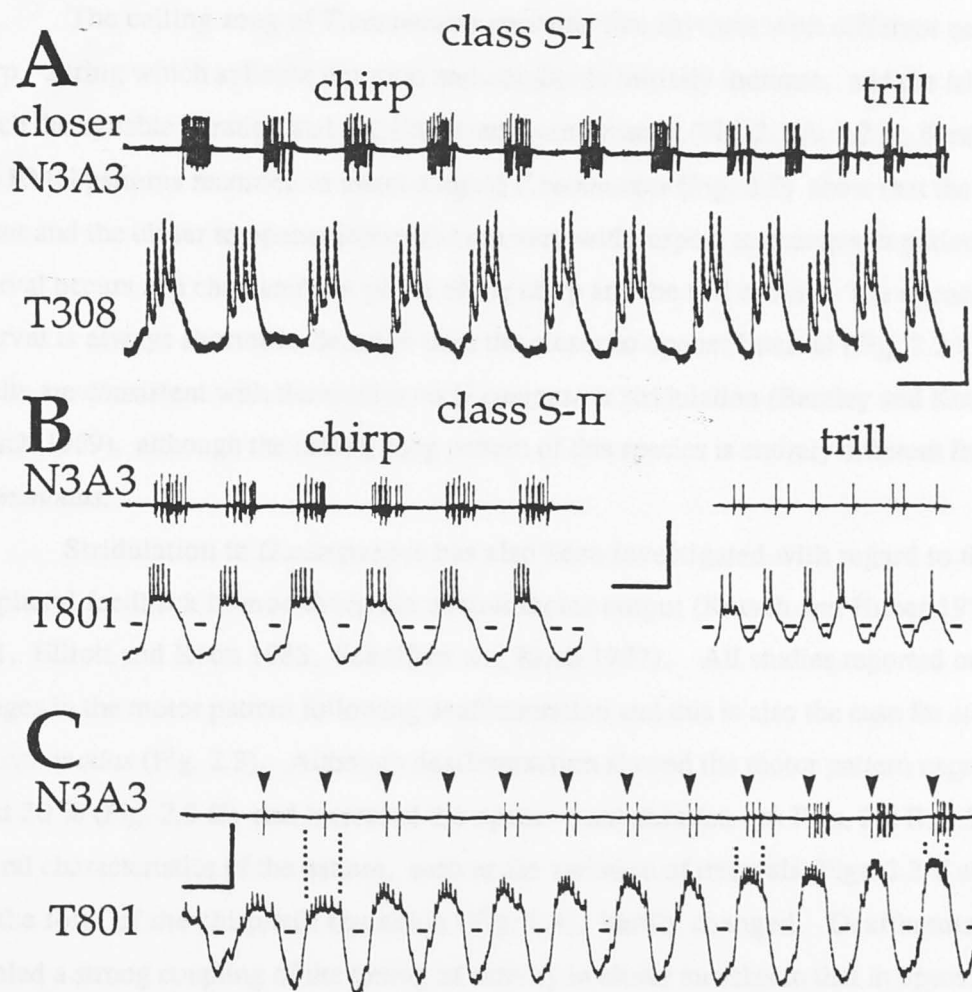


Fig. 2.9 A - C. Recordings from representatives of the two physiological classes of interneurons formed according to the timing of their bursts during the stridulatory cycle. **A** *Class S-I*: Burst occurring in antiphase to the closer activity recorded extracellularly from mesothoracic nerve 3A3. Example from interneuron T308 (see Fig. 2.8). **B**, **C** *Class S-II*: Burst starting during the opener phase and ending during the closer phase. Example from interneuron T801 (see Fig. 2.8). **C** The amplitude of the excitation in T801 as well as the duration of the closer discharge increase while the cycle periods during the chirp stay constant (as indicated by arrowheads at the beginning of a burst in N3A3). Calibration: Vertical **A**, **C** 10 mV **B** 20 mV; Horizontal **A**, **B**, **C** 50 ms

5. DISCUSSION

5.1. Electromyograms and the effects of deafferentation

The calling song of *T.commodus* contains two rhythms with different periods: the chirp, during which syllable duration and amplitude initially increase, and the trill, during which the syllable duration and amplitude are more constant (Fig. 2.1 A, 2.2 A; Bentley 1971). The EMG patterns recorded in intact singing *T.commodus* (Fig. 2.2) show that the opener to closer and the closer to opener intervals both vary with respect to changes in period and each interval occurs at a characteristic phase of the chirp and the trill cycles. The opener to closer interval is always shorter in duration than the closer to opener interval (Fig. 2.2 C). These results are consistent with the studies on *G.campestris* stridulation (Bentley and Kutsch 1966, Kutsch 1969), although the calling song pattern of this species is entirely different from that of *T.commodus*.

Stridulation in *G.campestris* has also been investigated with regard to the role of peripheral feedback in modifying the central motor output (Kutsch and Huber 1970, Möss 1971, Elliott and Koch 1983, Schöffner and Koch 1987). All studies reported only minor changes in the motor pattern following deafferentation and this is also the case for stridulation in *T.commodus* (Fig. 2.3). Although deafferentation slowed the motor pattern expression by about 20 % (Fig. 2.3 C) and increased the opener burst duration (cf. Figs. 2.2 B, 2.3 A), the general characteristics of the pattern, such as the variation of intervals (Figs. 2.2 C and 2.3 D) and the form of the chirp/trill transition (Fig. 2.4), hardly changed. Deafferentation also revealed a strong coupling of the timing of activity in closer muscles to that in opener muscles (Fig. 2.3 E). These results support Bentley's (1969a) report of a slower rhythm in deafferented preparations and the appearance of time locking which he termed "opener-closer burst couplets".

The observations by all investigators that deafferentation has only minor effects on stridulatory behaviour contrasts with the findings for other insect motor patterns such as locust flight (Wendler 1974, Pearson and Wolf 1987), stick insect walking (Bässler 1983), cockroach walking (Delcomyn 1984), and cockroach ventilation (Farley and Case 1968) where peripheral feedback was shown to significantly modify the centrally generated motor pattern. While the latter motor systems have to account for environmentally induced perturbations to which they adapt by integrating phasic sensory information, the motor pattern

generator for stridulation in the cricket may be able to rely almost entirely on central mechanisms in order to produce the species-specific timing of the syllables in the calling song.

5.2. Synaptic input to motoneurons

Bentley (1969a) presented intracellular recordings from opener and closer motoneurons during stridulation in *G.campestris*. In general his recordings (obtained from second basalar (opener) and promotor coxae (closer) motoneurons) reveal similar synaptic events occurring during stridulation to those reported here for subalar (opener) and remotor coxae (closer) motoneurons in *T.commodus*, although the stridulatory pattern of *G.campestris* does not contain the two different rhythms seen in *T.commodus* (Fig. 2.1 A). My investigation of the patterns of synaptic input to opener and closer motoneurons reveals that (1) the same motoneurons show different burst patterns during the chirp and the trill; and (2) there nevertheless appear to be synaptic components which occur during both the chirp and the trill. For opener motoneurons this synaptic component is a short but fast depolarisation (Fig. 2.6), for closer motoneurons it is an IPSP followed by an excitation (Fig. 2.7). These synaptic inputs can also be observed when the stridulatory pattern becomes irregular and they appear as "single events" during the recordings. Thus it seems they most likely originate from common interneurons which are activated during the chirp and the trill. However, the chirp rhythm is not simply a slowed version of the trill. The major changes occurring during the expression of the chirp rhythm consist of an accurately timed additional excitation following the initial action potential in the closer motoneuron burst (Fig. 2.7 D). This additional excitation is the basis for the chirp syllables which have a different amplitude and duration from those in the trill (Fig. 2.1 A, 2.2 A). This excitation may reflect the activity of an interneuron which accumulates excitation, possibly only at low repetition rates. This proposed interneuron is most likely part of the closer premotor pathway, rather than the oscillator, because these effects are only observed in closer motoneurons. The source of excitation to this interneuron could originate from the oscillator, or even from the closer motoneurons themselves. The neuronal mechanism responsible for switching the motor output from chirp to trill is independent of any accumulating excitation which might feed back from motoneurons (Fig. 2.7 E), or from peripheral receptors on the wing base or cerci (Fig. 2.4).

5.3. Interneuronal burst patterns

The somata of all seven interneurons identified in this study are located in the metathoracic ganglion and four of these interneurons have axons descending in the abdominal nerve cord. This is an interesting feature of the neuronal organisation of the stridulatory system in the cricket, since the motoneurons and muscles involved in stridulation are all located in the mesothoracic segment. Links have been proposed between the motor pattern generator for stridulation and other neuronal centres in the metathoracic and abdominal ganglia. For instance, the cerci may play an important role in providing sensory feedback to the stridulatory motor system (Dambach et al. 1983). Furthermore, the coupling of stridulatory and respiratory activity in crickets is also well documented (Huber 1960b, Janiszewski and Otto 1989). Hence, some of the interneurons reported in this study may provide the link from the stridulatory system to other systems.

None of the interneurons reported here resets the stridulatory rhythm or unequivocally switches the pattern from chirp to trill upon experimental current injection. Interneurons which exert their synaptic effects via a graded synaptic transmission have been reported for other motor systems (Pearson and Fournier 1975, Burrows and Siegler 1978, Graubard et al. 1983). To date non-spiking interneurons have not been found to be part of the mechanism by which the stridulatory motor pattern is generated. However, the participation of such graded interactions cannot be excluded. Thus, recordings from further interneurons are still needed to elucidate the cellular basis for rhythm generation during stridulation and to describe the neuronal mechanisms for switching between the two rhythms, the chirp and the trill.

The burst patterns of interneurons recorded so far are also not sufficient to explain the burst pattern seen in motoneurons (Fig. 2.10 A). Nevertheless, the discharge pattern of motoneurons allows one to predict the timing and burst patterns of at least some interneurons necessary for stridulation. (1) Closer motoneurons first receive an IPSP followed by a sharp depolarisation which is again followed by an additional excitation (Fig. 2.7). Thus, at least three types of specifically timed interneuronal burst may be expected (Fig. 2.10 B): one type should show a brief excitation at the time of the IPSP, while a second type should show a brief excitation for the initial phase of the closer discharge. The bursts of these two interneurons are likely to be coupled, since this pattern of inhibition and excitation in closer motoneurons appears to be linked in both the chirp and trill (Fig. 2.7). These two interneurons should not

change their burst pattern from chirp to trill, except for a change in cycle period. A third type of interneuron is required to maintain the excitation in closer motoneurons during a chirp. This type of interneuron may not be active during a trill. Only one type of interneuron was recorded which may account for the initial action potential in the closer burst (T801 in *class S-II*, Fig. 2.8; Fig. 2.9 B, C; Fig. 2.10 A). (2) Opener motoneurons show a tonic depolarisation upon which a phasic depolarisation is superimposed (Fig. 2.6, Fig. 2.10 C). To account for this, one interneuron should show an excitation in antiphase to the closer discharge and produce the general excitation seen in opener motoneurons at that time. This interneuron does not need to be active during the trill. A second interneuron which might shape the fast phasic depolarisation in opener motoneurons should be active in both the chirp and trill, and

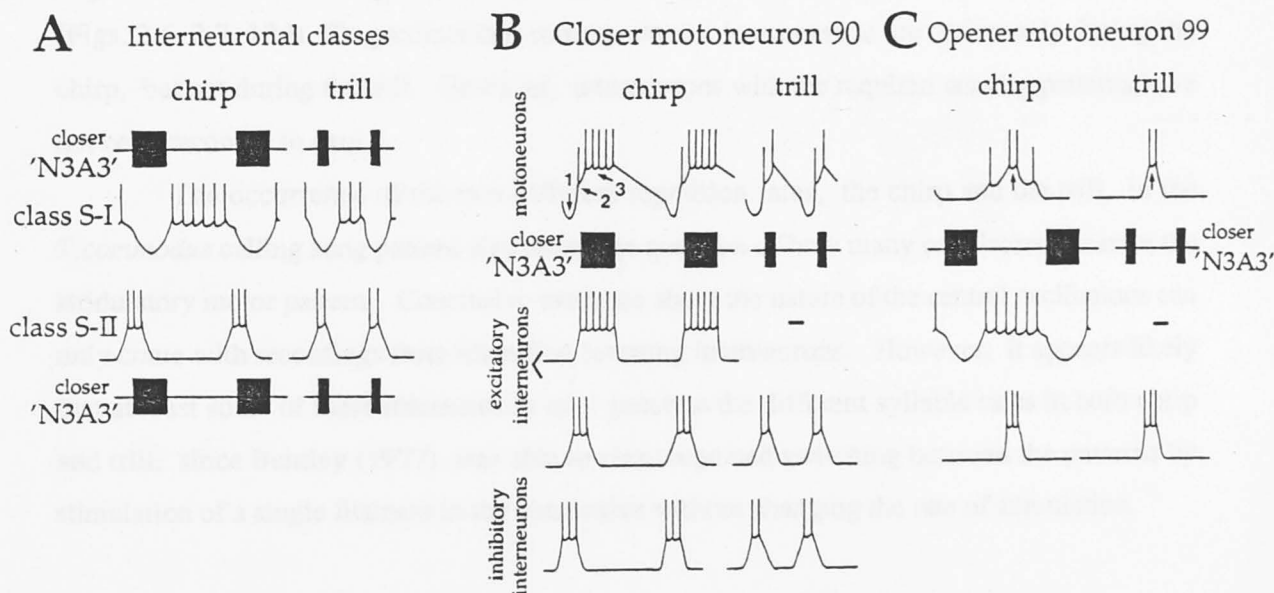


Fig. 2.10 A - C. Interneuronal discharge patterns and their relation to phases of activity in motoneurons. **A** Schematic presentation of the interneuronal burst patterns encountered in this study (see Fig. 2.9). **B** The activity of closer motoneurons during a chirp shows three phases, an initial IPSP (arrow 1), followed by a weak excitation (arrow 2), followed by an additional excitation (arrow 3). The last excitation does not occur during a trill. Below each motoneuronal burst the discharge patterns of interneurons which could account for the motoneuronal response are indicated. Three types of timing of interneuronal bursts may be predicted. Only one type is actually found (see A). **C** The activity of opener motoneurons during a chirp consists of a tonic and a superimposed phasic depolarisation (arrow). During the trill only the phasic depolarisation is present. Interneurons which could account for the tonic, but not for the phasic, discharge were recorded.

the excitatory input to this interneuron should also be invariant of the transition from chirp to trill (Figs. 2.6 and 2.10 C). These two interneurons should both have excitatory effects on the motoneurons. Interneurons in *class S-I* show the appropriate timing to account for the general excitation seen in opener motoneurons (Figs. 2.6, 2.8 A, 2.10 C), but all the interneurons recorded were active during the chirp as well as during the trill (Fig. 2.8 A). An interneuron of the second required type, though predicted, has not been found to date.

To summarise, there are two major predictions about the timing of interneuronal bursts as inferred from the motoneuronal discharge: (1) Some of the synaptic inputs to motoneurons stay constant during chirp and trill (Figs. 2.6 and 2.7), which implies that the burst pattern of some interneurons should not change at the transition from chirp to trill except for the cycle duration (Fig. 2.10 B, C). (2) The pattern of synaptic input to motoneurons (Figs. 2.6, 2.7, 10 B, C) predicts that some premotor interneurons are active only during the chirp, but not during the trill. However, interneurons with the required activity patterns have not been recorded to date.

The occurrence of the two different repetition rates, the chirp and the trill, in the *T.commodus* calling song pattern also raises the question of how many oscillators generate the stridulatory motor pattern. Conclusive evidence about the nature of the central oscillations can only come with recordings from identified resetting interneurons. However, it appears likely that at least some of these interneurons may generate the different syllable rates in both chirp and trill, since Bentley (1977) was able to elicit repeated switching between the patterns by stimulation of a single filament in the connective without changing the rate of stimulation.

CHAPTER 3

NEURONAL ORGANISATION OF THE FLIGHT MOTOR PATTERN IN THE CRICKET, *TELEOGRYLLUS COMMODUS*

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1. SUMMARY

The neuronal organisation of the flight motor pattern in the cricket was investigated using EMG and intracellular recording and staining techniques with two objectives: (a) to compare neuromuscular control of the forewings during cricket flight and stridulation, and (b) to provide data for comparison with the well characterised locust flight system.

1. A cricket suspended in a wind stream shows either active, wing-beating, flight or a wing vibration. The cycle periods for each condition are different, however, the motor patterns appear to be very similar (Fig. 3.1). After deafferentation, cycle periods increase significantly and so does the variability of the centrally generated motor pattern (Fig. 3.2).

2. Meso- and metathoracic elevator motoneurons (90, 119) show very similar patterns of synaptic input which may be divided into three phases: a slow depolarisation, a fast depolarisation, and a repolarisation supported by IPSPs at the time of depressor activity (Figs. 3.4 and 3.6). By contrast, mesothoracic subalar MN 99 and metathoracic subalar MN 129, both of which are depressors, receive different patterns of synaptic input. MN 129 receives a short, phasic depolarisation which may be preceded by an IPSP, whereas MN 99 shows a more tonic depolarisation interrupted by a distinct IPSP at the time of hindwing depressor activity (Figs. 3.5 and 3.7).

3. 12 flight interneurons were identified (Fig. 3.8), in addition to the 5 interneurons described previously by Robertson (1987). These 17 interneurons may be grouped into four classes according to the timing and the shape of their bursts in a flight cycle (Fig. 3.9). This grouping of interneurons allows us to account for the timing of synaptic input which motoneurons receive during flight and suffices to explain some features of the flight motor pattern such as the elevator to depressor coupling (Fig. 3.12).

4. Interneurons T401 (Fig. 3.10) and T404 (Fig. 3.11) play important roles in flight rhythm generation (resetting and initiation, respectively) in the cricket and the locust. The findings of this study together with a previous one (Robertson 1987) suggest a similar organisation of the basic circuitry generating the flight motor pattern in cricket and locust.

2. INTRODUCTION

The forewings and their associated musculature are involved in two distinct behaviours in crickets, namely flight and stridulation. A functional description of neuronal circuits controlling wing movements during these behaviours will allow us to answer questions such as whether the pattern generating neuronal circuits are shared or separate and how switching occurs between the two behaviours. In order to address these questions adequately the neuronal organisation of each motor pattern must first be described. This has been done for the stridulatory motor pattern (Kutsch 1969, Bentley 1969a, 1971, Chapter 2), but there are still considerable gaps in the description of the flight motor pattern in the cricket.

The basic flight rhythm is produced at a wing beat frequency of approximately 25 - 30 Hz (Kutsch 1969, Bentley 1969b, Möss 1971), and consists of alternating activity in depressor and elevator muscles of both pairs of wings (Huber 1960a, Kutsch 1969, Bentley and Hoy 1970) with activity in hindwing muscles leading that in the forewing muscles (Bentley and Hoy 1970, Robertson 1987). The role of peripheral input in generating flight motor activity is not clear. Möss (1971) did not observe changes in the flight rhythm after deafferentation of thoracic proprioceptors, whereas Bentley (1969b) noted a slowing of the flight frequency. Robertson (1987) has shown that deafferentation affects the generation of the motor pattern in the cricket in a similar way to that in the locust flight system, namely by a decrease in the flight frequency (Wilson 1961, Wilson and Gettrup 1963, Robertson and Pearson 1982).

The first account of intracellularly recorded activity of cricket motoneurons during flight is by Bentley (1969b). Robertson (1987) identified the first cricket interneurons able to reset the flight rhythm upon experimental current injection and also demonstrated that some of these interneurons exist as serial homologs in the fused neuromeres comprising the metathoracic ganglion.

The goal of the present study is to provide a description of motoneuronal and interneuronal activity during the expression of the flight rhythm in the cricket. The motoneurons chosen for study were selected in order to allow comparisons with previous work on the flight (Kutsch 1969, Bentley and Hoy 1970, Möss 1971) and the stridulatory rhythm (Bentley and Kutsch 1966, Kutsch 1969, Chapter 2). The interneurons reported in the present study confirm and extend Robertson's (1987) findings and allow specific comparisons with the neuronal control of flight in another orthopteran insect, the locust.

3. MATERIALS AND METHODS

3.1. Animals

Adult crickets, *Teleogryllus commodus* (Walker), were caught near Canberra, Australia, or taken from a breeding colony not more than two generations old. No difference in the flight performance of crickets from the two sources was detected. Crickets of both sexes were used, however, sex specific effects were evident during adult life. In adult female *Teleogryllus* all flight muscles appear to degenerate with age (Ready and Josephson 1982, Ready and Najm 1985, Robertson 1987), whereas the males retain the full strength of those mesothoracic muscles which are also used during stridulation (unpublished observation). As a result, in order to avoid age-dependent effects, crickets which had been adult for no more than 10 days were used for electromyographic analysis (EMG). Despite the dramatic change in muscle structure with age and between sexes, no consistent differences in the flight motor pattern evoked for intracellular analysis were noted.

3.2. Preparation and recording

All legs were removed and the cricket was then pinned dorsal side up onto a small cork platform which did not restrain wing movements. Electrodes (50 μ m silver wire, insulated except at the tip) were inserted through a small hole in the cuticle into forewing muscles 90 and 99 and hindwing muscles 119 and 129, and the indifferent electrode placed in the abdomen. These muscles were selected for study because they allow a direct comparison with previous studies on *Gryllus campestris* (Kutsch 1969, Bentley and Hoy 1970, Möss 1971). Nomenclature of muscles and nerves follows Kutsch (1969) and Kutsch and Huber (1970), respectively. The metathoracic nerve 1 separates very early after exiting the ganglion forming branches 1A and 1B. Nerve 1B splits to branches 1C and 1D; the latter containing motor axons which innervate the metathoracic dorsal longitudinal muscle (Kutsch and Huber 1970). Nerve branches 1A and 1B correspond to roots 1 and 2, respectively, of Furukawa et al. (1983) and Robertson (1987). Usually only two muscles were recorded at a time in any one animal. Flight was elicited either by a windstream directed towards the head from anterior, or a brief wind puff onto the cerci. Many animals also had a suction electrode placed

on their brain which allowed stridulation to be elicited. No differences in the flight motor pattern elicited by these various methods were observed (Hennig 1988).

The preparation for intracellular recording was the same as previously described (Robertson 1987; Chapter 2). To monitor ongoing flight activity metathoracic nerve 1D, which contains axons of motoneurons that innervate the hindwing dorsal longitudinal muscle (a depressor), was lifted onto a 75 μm silver wire hook electrode. In many preparations mesothoracic nerve 3A3 innervating forewing muscle 89, an elevator, was also monitored. All peripheral thoracic nerves were cut in order to abolish rhythmical muscle activity and to exclude feedback from thoracic proprioceptors. TES buffered saline was used to keep the preparation moist (Chapter 2).

Standard techniques were employed in order to record intracellularly from motoneurons and interneurons, and to amplify, display, store, and play back the recorded nervous activity (Chapter 2). Similarly, the dye filling of recorded cells with Lucifer Yellow followed methods described in chapter 2. To characterise the flight motor pattern in terms of activity in identified motoneurons, mesothoracic motoneurons 90 and 99 (elevator and depressor of the forewing, respectively) and metathoracic motoneurons 119 and 129 (elevator and depressor of the hindwing, respectively) were intracellularly recorded, as these neurons have been previously described in both *G.campestris* (Elepfandt 1980, Elliott 1983), *Teleogryllus oceanicus* (Wang and Robertson 1989), and *T.commodus* (Chapter 2). Interneurons were recorded in the mesothoracic and metathoracic ganglia, and only data from interneurons which were identified by dye filling after recording their physiology are included in this study. Interneurons were identified according to the scheme proposed by Robertson (1987; see also chapter 2). Robertson's (1987) recordings from flight interneurons were obtained on the cricket *T.oceanicus*, while this study used *T.commodus*, however no species-specific differences in the flight motor pattern between these two closely related crickets were found.

4. RESULTS

4.1. Flight or vibrational flight ?

An intact cricket suspended in a wind current starts either active, wing-beating, flight or a wing vibration ("Vibrationsflug") described for *G.campestris* by Huber (1960a, Kutsch 1969, Möss 1971) and for *Teleogryllus* by Nolen and Hoy (1986, Moiseff et al 1978,

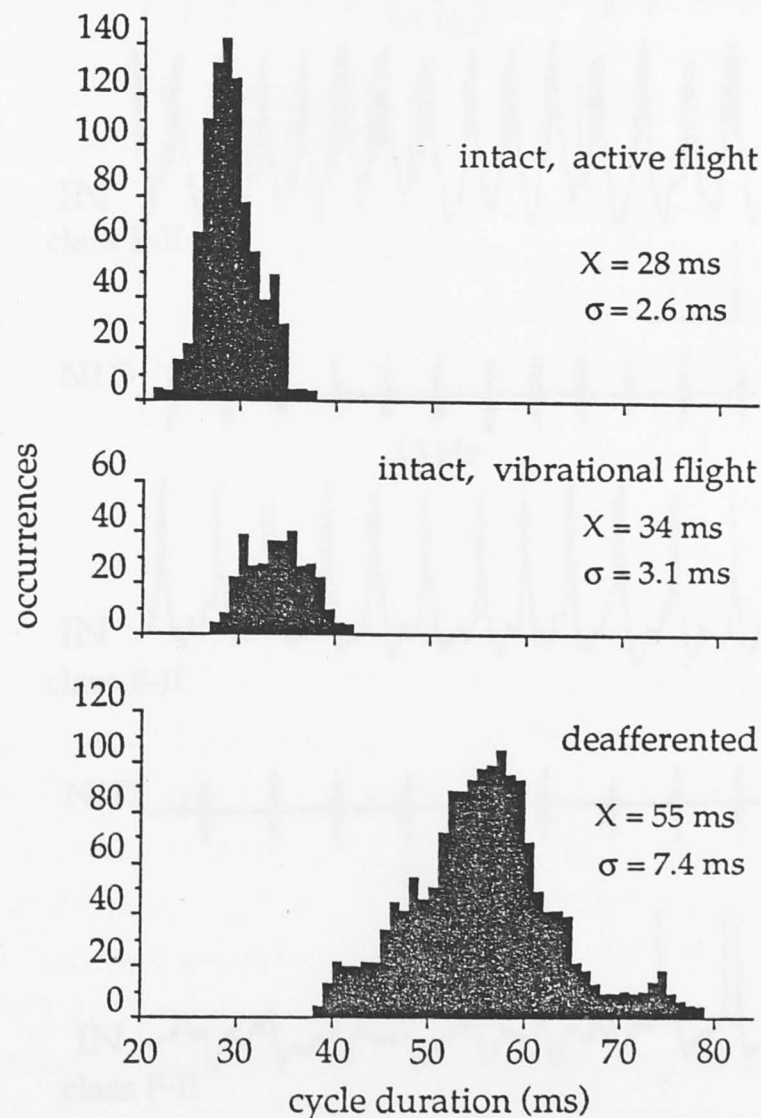


Fig. 3.1. Variations in the flight motor rhythm in intact and deafferented crickets. Histograms of cycle durations measured under different conditions: Intact, wing flapping flight (upper histogram), intact vibrational flight (middle histogram), and the deafferented flight pattern (bottom histogram). The mean values as well as the standard deviations are different for all three histograms.

Tomioka and Yamaguchi 1980, Robertson 1987) which is assumed to involve the same motor pattern as during actual wing beating flight. However, a consistent difference between active and vibrational flight is the wing beat frequency (Fig. 3.1). During active flight, period durations (depressor to depressor burst) measure 28.4 ms (\pm 2.6 ms) whereas during vibrational flight they are higher (33.6 ms \pm 3.1 ms) indicating a slower rhythm. In a given

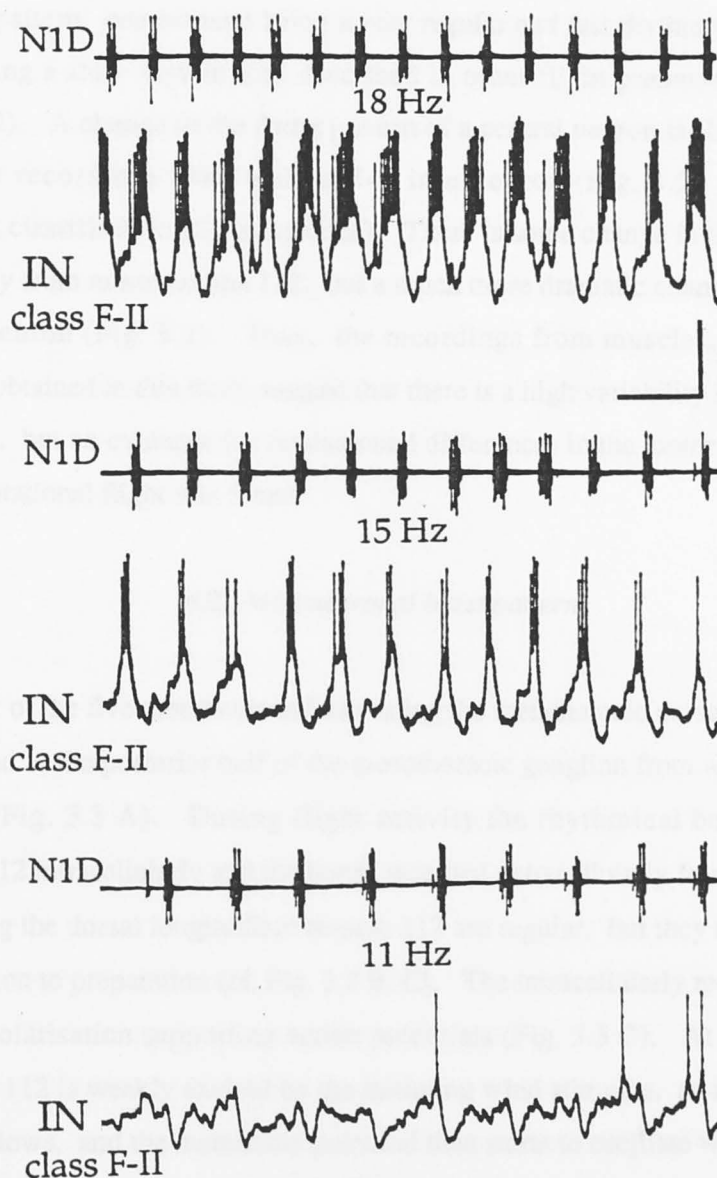


Fig. 3.2. Variations in the flight motor rhythm in deafferented crickets. Recording of a metathoracic interneuron (class F-II: see Fig. 3.9) which shows the range of cycle durations and discharge patterns in a single flight sequence (early in the flight sequence: upper trace (cycle duration \sim 50 ms i.e. 18 Hz); middle of the flight sequence: middle trace (cycle duration \sim 65 ms i.e. 15 Hz); end of flight sequence: bottom trace (cycle duration \sim 85 ms i.e. 11 Hz). Calibration: Vertical 10 mV; Horizontal 100 ms.

individual, vibrational flight also shows less variation in wing beat frequency than during active flight. The appearance of active flight or vibrational flight could not be correlated to different behavioural contexts or muscular conditions. In deafferented preparations it was usually not possible to distinguish these two modes of wing movement as deriving from different motor patterns solely on the basis of their different period durations (Fig. 3.1, bottom histogram). This is because deafferented preparations exhibit considerable variation in the flight motor pattern, one extreme being a very regular and fast rhythm (see Robertson 1985), the other being a slow rhythm also described in other flight preparations (Robertson and Pearson 1982). A change in the firing pattern of a central neuron is also seen in the case of intracellular recordings from a class F-II interneuron (Fig. 3.2; see Fig. 3.9 for the physiological classification of interneurons). There is some change in the burst rate recorded extracellularly from motoneurons 112, but a much more dramatic change in the burst pattern of the interneuron (Fig. 3.2). Thus, the recordings from muscles, motoneurons, and interneurons obtained in this study suggest that there is a high variability in some aspects of the motor pattern, but no evidence for fundamental differences in the motor pattern generation of active and vibrational flight was found.

4.2. Motoneuronal burst patterns

Four of the five motoneurons innervating the metathoracic dorsal longitudinal muscle 112 are located in the posterior half of the mesothoracic ganglion from which their axons exit via nerve 6 (Fig. 3.3 A). During flight activity the rhythmical bursts recorded from motoneuron 112 intracellularly and the bursts recorded extracellularly from metathoracic nerve 1D innervating the dorsal longitudinal muscle 112 are regular, but they may vary in duration from preparation to preparation (cf. Fig. 3.3 B, C). The intracellularly recorded burst consists of a brief depolarisation supporting action potentials (Fig. 3.3 C). At the onset of a flight sequence MN 112 is weakly excited by the initiating wind stimulus, an inhibition typical for depressors follows, and the membrane potential then starts to oscillate with the flight rhythm (not shown). Extracellular recordings of these 5 motoneurons always showed a regular bursting activity during flight and were therefore used as a reliable monitor for ongoing flight activity.

The mesothoracic remotor coxae motoneuron 90, an elevator of the forewing during flight, shows a strong, often tonic, initial depolarisation to a wind stimulus before oscillations

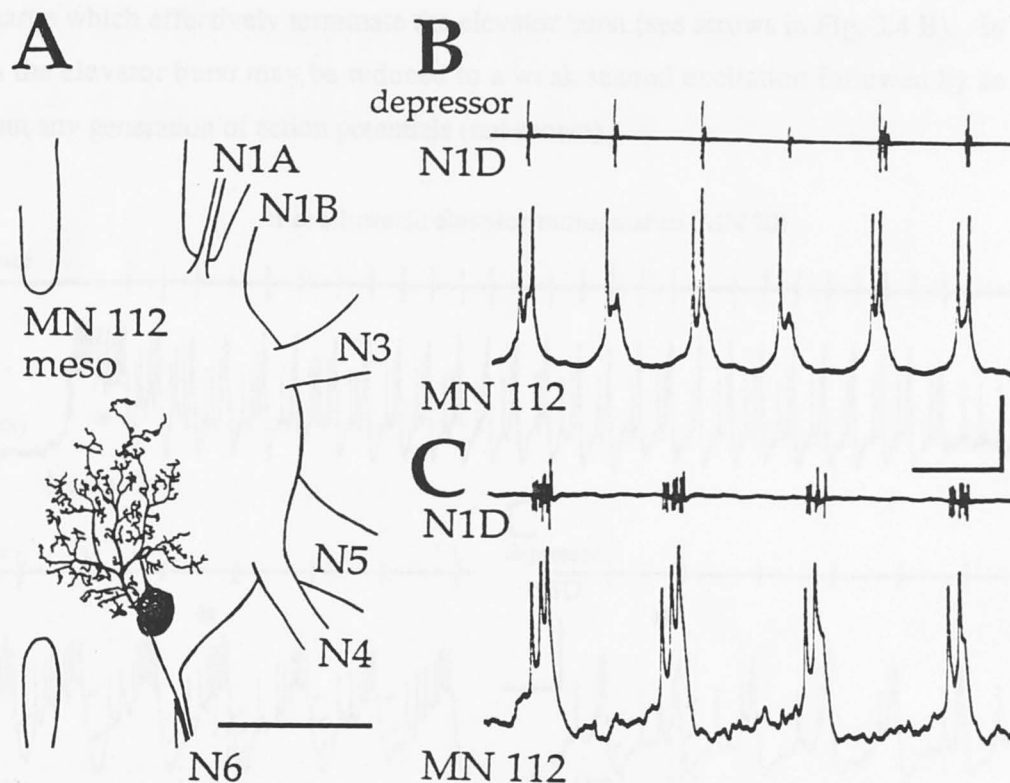


Fig. 3.3 A - C. Hindwing depressor motoneuron MN 112 which innervates the metathoracic dorsal longitudinal muscle, and its activity during flight. **A** The morphology of MN 112 in the mesothoracic ganglion as seen in wholemount following intracellular staining with Lucifer Yellow. Scale: 200 μ m. **B, C** Two examples of recordings from different animals which show the range of variability encountered in the extra- and intracellularly recorded burst pattern of MN 112. The intracellular recording in **B** is taken contralateral to the extracellularly recorded bursts. Calibration: Vertical **B, C** 20 mV; Horizontal **B, C** 50 ms.

of the membrane potential coupled to the flight rhythm commence (Fig. 3.4 A). The burst pattern of MN 90 during a flight cycle may be divided into three phases. Initially a slowly increasing depolarisation generates a variable number of action potentials (cf. cycles marked with an asterisk in Fig. 3.4 B, C). This depolarisation is strongest during the first cycles of a flight sequence and then gradually weakens, but it may be restored by a transient wind stimulus. The second phase of the burst consists of a strong phasic depolarisation generating two or three action potentials just prior to the discharge of the metathoracic dorsal longitudinal muscle (Fig. 3.4 C). This depolarisation is also present in the initial cycles of the sequence, although there it is often indistinguishable from the first depolarisation (Fig. 3.4 B). The third

phase of the burst consists of several IPSPs occurring at the time of dorsal longitudinal discharge which effectively terminate the elevator burst (see arrows in Fig. 3.4 B). In some cases the elevator burst may be reduced to a weak second excitation followed by an IPSP without any generation of action potentials (not shown).

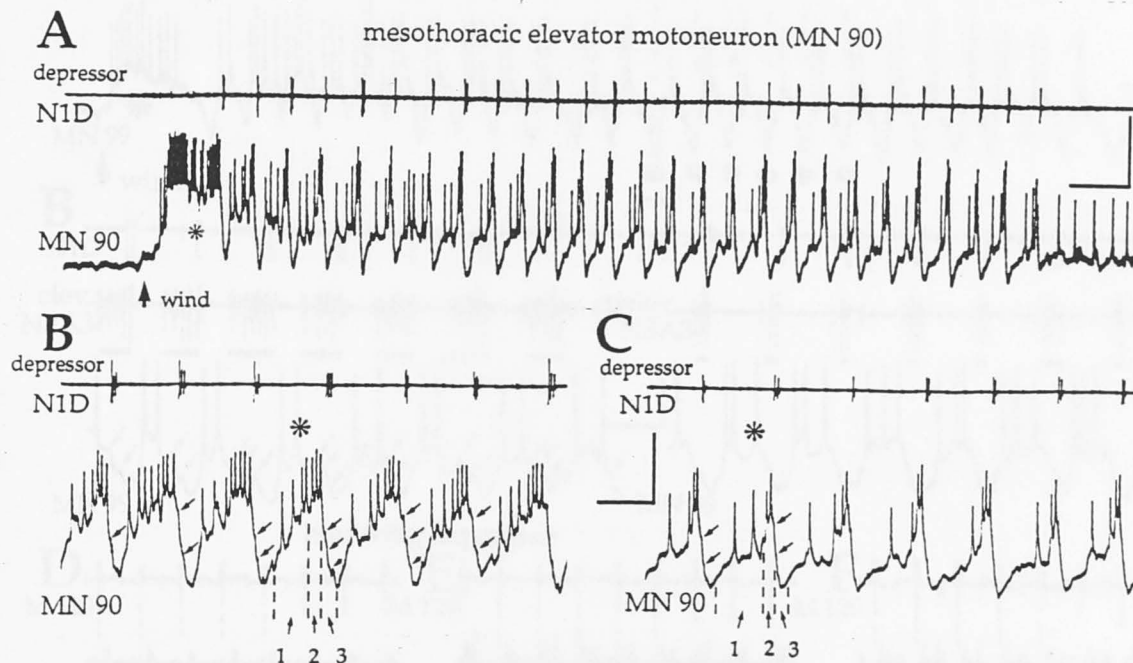


Fig. 3.4 A - C. Pattern of synaptic input to mesothoracic elevator motoneuron 90 during a flight sequence. **A** A flight sequence commences with a tonic, wind induced depolarisation (asterisk) before rhythmical oscillations in membrane potential start to occur. **B, C** The synaptic input during a flight cycle may be divided into three phases. (1) A slow depolarisation which generates a variable number of action potentials (cf. bursts marked with an asterisk in **B** and **C**). (2) Then a sharp depolarisation just prior to the depressor burst recorded in nerve 1 D (N1D) which is followed (3) by a fast repolarisation supported by IPSPs (arrows in **B** and **C**). Calibration: Vertical **A - C** 10 mV; Horizontal **A** 100 ms **B, C** 50 ms.

The mesothoracic subalar motoneuron 99, a depressor of the forewing during flight, exhibits an initial depolarisation upon a flight initiating wind stimulus similar to the one described for MN 90 (Fig. 3.5 A). The oscillation in membrane potential during flight in MN 99 consists of an excitation followed by IPSPs at the time of metathoracic dorsal longitudinal discharge (Fig. 3.5 A). The excitation generating action potentials is more tonic than phasic and often lasts until the IPSPs terminate the burst. Thus, MN 99 often appears rather like an elevator because of its seemingly antagonistic activity to the dorsal longitudinal burst, a

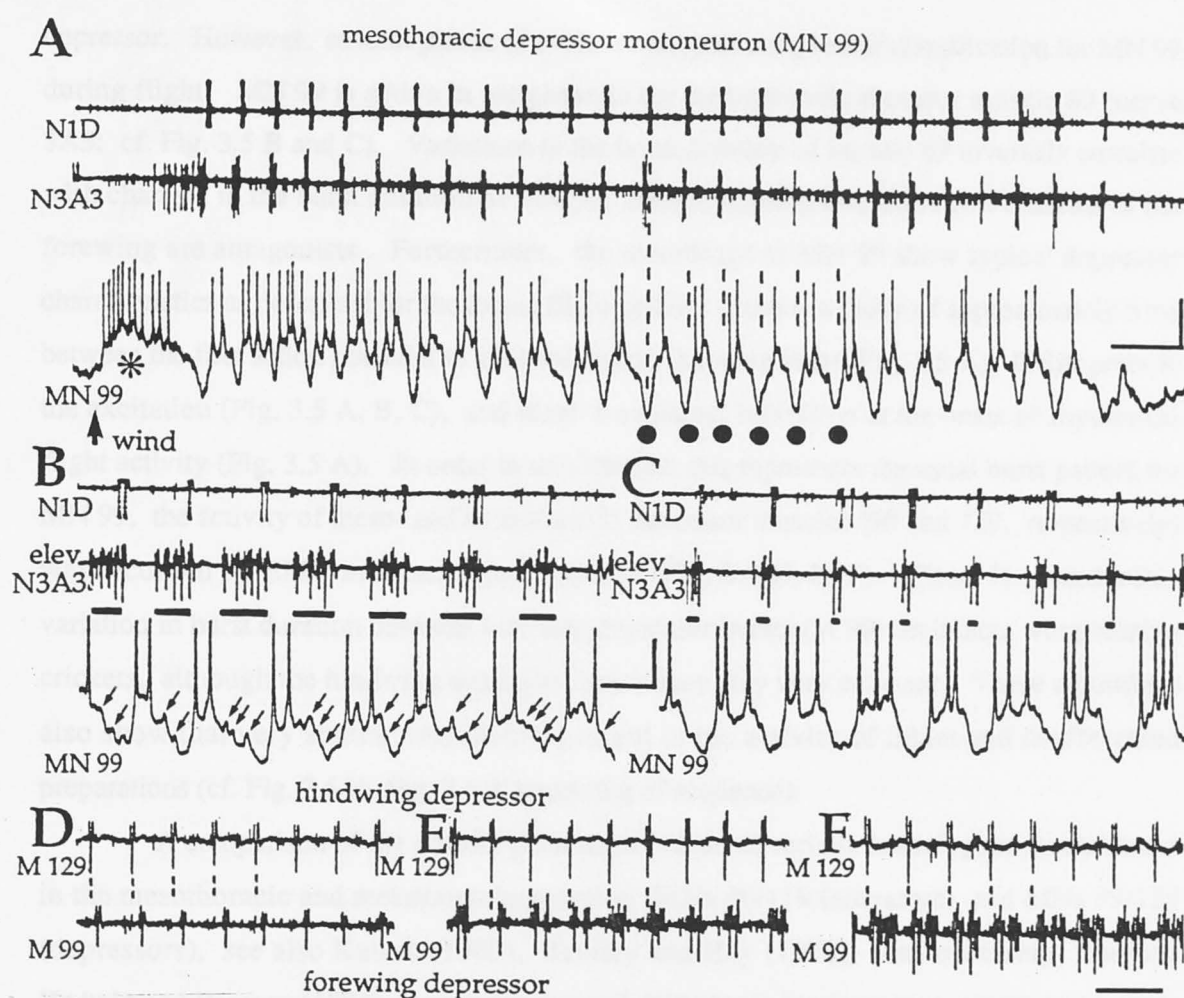


Fig. 3.5 A - F. Pattern of synaptic input to mesothoracic depressor motoneuron 99 during a flight sequence. **A** A flight sequence begins with a tonic depolarisation (asterisk) upon a wind stimulus. At hindwing depressor activity (upper trace: extracellular recording of metathoracic nerve 1D (N1D), its bursts are indicated by a filled circle and the dashed line), MN 99 receives an IPSP which interrupts the only weakly phasic excitation. The first action potential in a MN 99 burst occurs approximately 5 - 10 ms after the hindwing depressor burst (dashed line). **B, C** MN 99 and the mesothoracic elevator muscle 89 (recorded in nerve 3A3 in the mesothoracic segment) show an antagonistic burst pattern. **B** Extended bursts in elevator M 89 (N3A3, the bursts are marked by the black lines) correspond to shorter MN 99 bursts (the IPSPs in the intracellular recording of MN 99 which suppress spiking activity are indicated by arrows). **C** Short bursts in elevator M 89 (N3A3) correlate to extended spiking activity in MN 99. **D, E, F** Variation in burst pattern of MN 99 also occurs in EMGs of muscle 99 recorded in intact flying crickets (upper trace: Metathoracic subalar muscle 129, a depressor of the hindwing; lower trace: mesothoracic subalar muscle 99, a depressor of the forewing). While M 129 shows no variation in its burst pattern, M 99 bursts may contain from one up to four action potentials. Dashed lines indicate the hindwing to forewing lag. Calibration: Vertical **A - C** 10 mV **D, E, F** the amplitude of the EMG potentials is uncalibrated; Horizontal **A** 100 ms **B, C** 40 ms **D, E, F** 50 ms.

depressor. However, several pieces of evidence support a depressor classification for MN 99 during flight. MN 99 is active in antiphase to the mesothoracic elevator muscle 89 (nerve 3A3; cf. Fig. 3.5 B and C). Variations in the burst duration of muscle 89 inversely correlate with changes in the burst duration of MN 99, thus indicating that these two muscles of the forewing are antagonists. Furthermore, the recordings of MN 99 show typical depressor characteristics as described for the locust flight system: there is a delay of approximately 5 ms between the first action potential in a hindwing and forewing burst (Fig. 3.5 A), IPSPs precede the excitation (Fig. 3.5 A, B, C), and there is a distinct inhibition at the onset of rhythmical flight activity (Fig. 3.5 A). In order to confirm that this represents the usual burst pattern for MN 99, the activity of meso- and metathoracic depressor muscles (99 and 129, respectively) was recorded by EMG in intact flying animals (Fig. 3.5 D, E, F). There is considerable variation in burst duration between forewing depressor bursts (M 99) in intact, wing-beating crickets, although the hindwing depressor bursts may stay very constant. These recordings also show that very similar variation is present in the activity of intact and deafferented preparations (cf. Fig. 3.5 F; Fig. 3.5 A beginning of sequence).

A comparison of the activity patterns of presumed serially homologous motoneurons in the mesothoracic and metathoracic ganglion (MNs 90/119 (elevators) and MNs 99/129 (depressors), see also Kutsch (1969), Bentley and Hoy (1970)) was necessary, because Hedwig and Pearson (1984) reported segmental differences for depressor motoneurons, as is also suggested here (Fig. 3.5 D, E, F). Furthermore, a description of the activity of metathoracic motoneurons is an important prerequisite for the later comparison of flight and stridulatory motor activity. Intracellular recordings were therefore made from MN 119 (an elevator and serial homologue of MN 90; Fig. 3.6 A, B) and MN 129 (a depressor and serial homologue of MN 99; Fig. 3.7 A, B) singly, and simultaneously with their mesothoracic homologue (MN 90 and MN 99). The basic burst pattern of MN 119 corresponds to that described for its mesothoracic homologue. The discharge pattern may again be divided into three components, and a flight sequence commences with an initial depolarisation before cyclic oscillations start (Fig. 3.6 C). The hindwing elevator burst commonly occurs just before the dorsal longitudinal discharge, thus dividing the depressor to depressor cycle unevenly (Fig. 3.6 D). The hindwing elevator burst usually leads the forewing elevator burst by 5 - 10 ms (Fig. 3.6 D), although, in some preparations they may be concurrent.

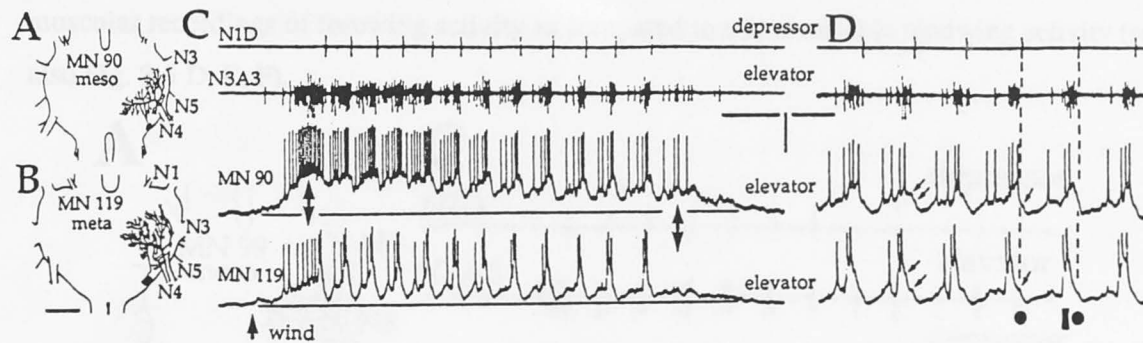


Fig. 3.6 A - D. Comparison of simultaneously recorded burst patterns during flight in presumably homologous elevator motoneurons in the mesothoracic (MN 90) and metathoracic ganglion (MN 119). **A, B** Lucifer Yellow fills of MN 90 (**A**) in the mesothoracic ganglion and MN 119 (**B**) in the metathoracic ganglion. Scale: 200 μ m. **C** A flight sequence showing similarities in the burst pattern of the two MNs at the beginning and the end of the sequence (double headed arrows). Traces: Upper: Metathoracic nerve 1D innervating the dorsal longitudinal muscle, a depressor of the hindwing; second trace: Mesothoracic nerve 3A3 innervating muscle 89, an elevator of the forewing; third and fourth trace: intracellular recordings of MN 90 and MN 119, respectively. **D** An expanded section of such a recording indicates the same burst pattern occurring in both MNs (see repolarisation phase and the IPSPs indicated by arrows). The elevator discharge (indicated by a black rectangle) divides the depressor to depressor cycle (indicated by a filled circle and dashed lines) unevenly. Calibration: Vertical **C, D** MN 90: 30 mV, MN 119: 40 mV; Horizontal **C** 100 ms **D** 50 ms.

The pattern of synaptic input to the metathoracic subalar MN 129 is in general similar to the one described for MN 112. A depolarisation supporting action potentials occurs at the time of the extracellularly recorded depressor burst (Fig. 3.7 C) and is often preceded by an IPSP (not shown). A flight sequence starts with a weak wind induced depolarisation, followed by rhythmical bursting (Fig. 3.7 C). Unlike the similar synaptic input to the various elevator motoneurons (90, 119; Fig. 3.6) the synaptic input to the serially homologous depressor motoneurons in the meso- and metathoracic ganglion is similar only with respect to the start of a flight sequence and the IPSP (not visible in this particular recording of MN 129, Fig. 3.7 D, E) preceding the first action potential of a burst. The synaptic input generating action potentials, however, is quite different (Fig. 3.7 D, E). Whereas in MN 129 the excitation is phasic, MN 99 receives an almost tonic depolarisation (Fig. 3.7 D), although the first action potential of a burst always occurs shortly after the beginning of the depolarisation. Figs. 3.7 D, E also exemplify the generally greater variation observed in motoneuronal and

muscular recordings of forewing activity as compared to a less variable hindwing activity (see also Fig. 3.5 D, E, F).

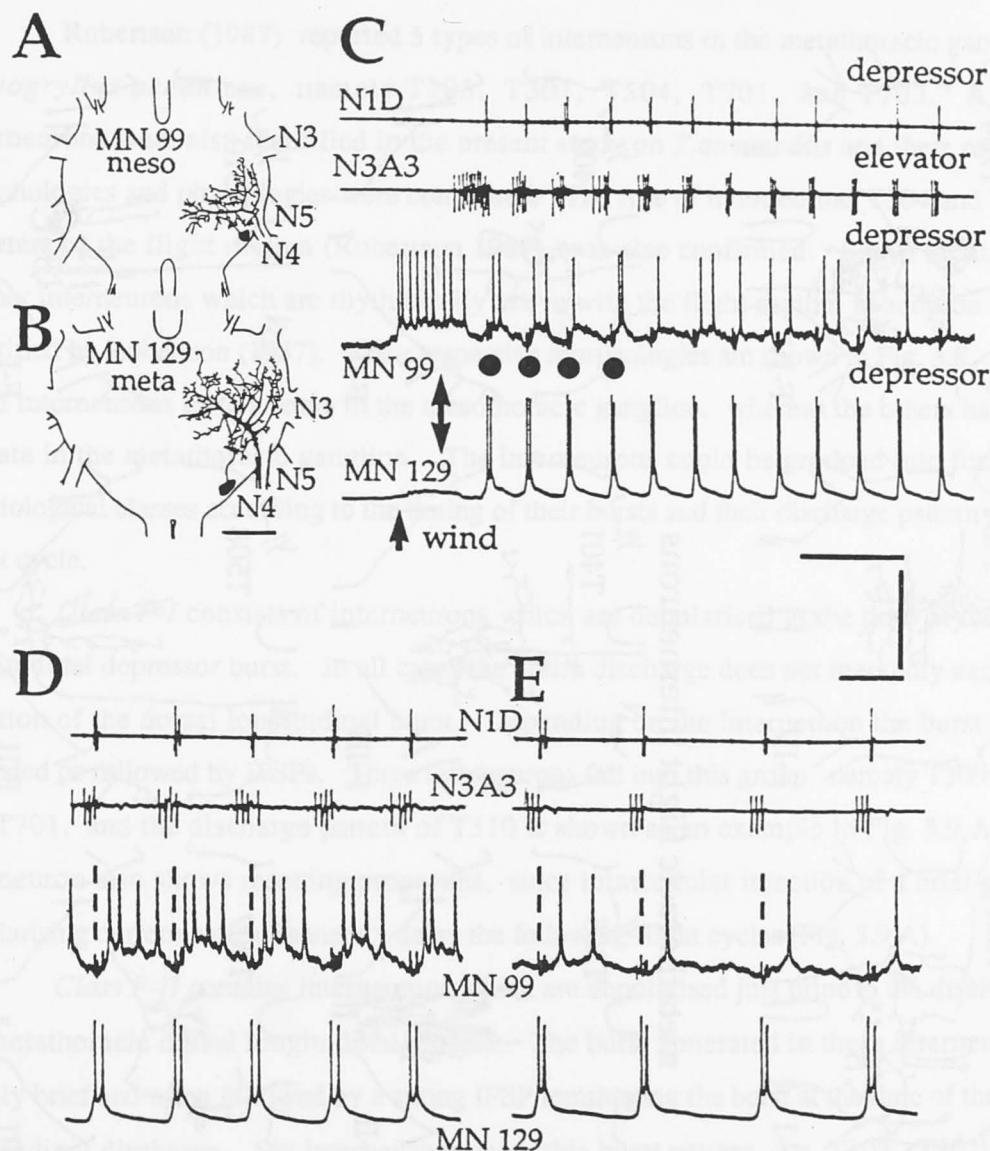
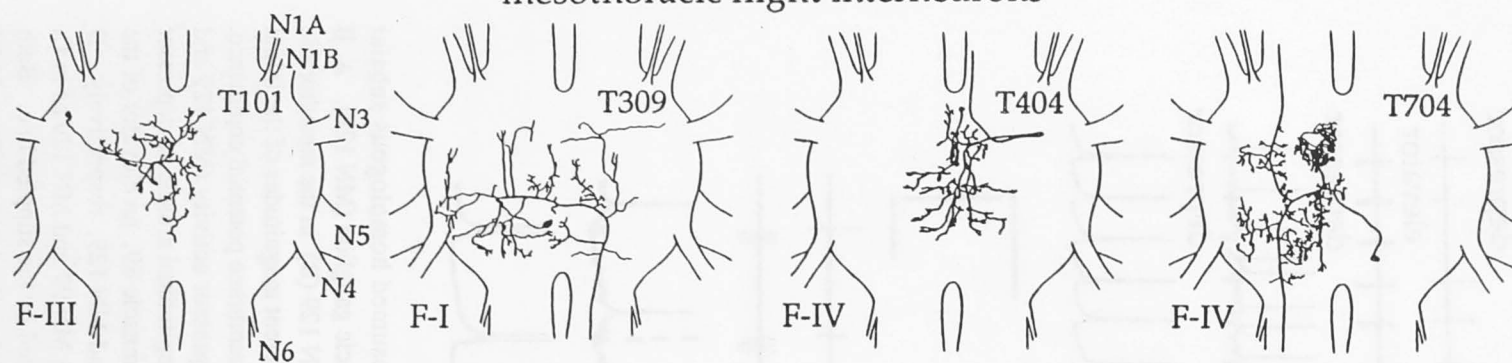
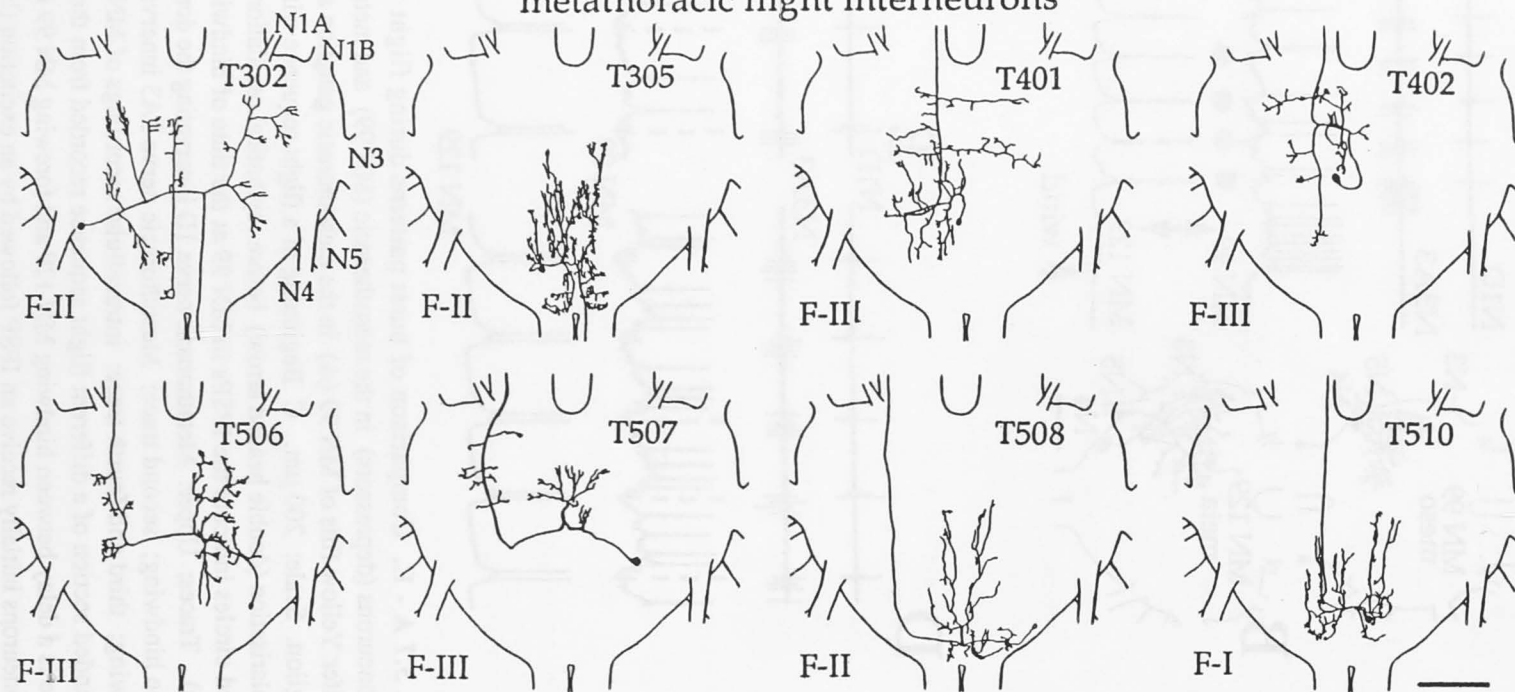


Fig. 3.7 A - E. Comparison of burst patterns during flight in presumed homologous subalar motoneurons (depressors) in the mesothoracic (MN 99) and metathoracic ganglion (MN 129). **A, B** Lucifer Yellow fills of MN 99 (**A**) in the mesothoracic ganglion and MN 129 (**B**) in the metathoracic ganglion. Scale: 200 μ m. **C** Beginning of a flight sequence with different magnitudes of the initial depolarisation (double headed arrow) before rhythmical oscillations in membrane potential commence. Filled circles indicate the IPSPs in MN 99 at the time of hindwing depressor activity (MN 129 and N1D). Traces: Upper: Metathoracic nerve 1D innervating the dorsal longitudinal muscle, a depressor of the hindwing; second trace: Mesothoracic nerve 3A3 innervating muscle 89, an elevator of the forewing; third and fourth trace: intracellular recordings of MN 99 and MN 129, respectively. **D** Expanded section of a different flight sequence recorded from the same MN 99 and MN 129 as in **C**. There is a delay between hindwing MN 129 and forewing MN 99 indicated by the stippled line. Both motoneurons initially receive an IPSP followed by an excitation (less obvious in this recording of MN 129). **E** Expanded section from the recording in **C**. While the excitation to MN 99 changes as compared to **D**, MN 129 does not follow the changes of synaptic input seen in MN 99. Calibration: Vertical **C, D, E** MN 99: 30 mV, MN 129: 50 mV; Horizontal **C** 200 ms **D, E** 50 ms.

mesothoracic flight interneurons



metathoracic flight interneurons



4.3. Interneuronal burst patterns

Robertson (1987) reported 5 types of interneurons in the metathoracic ganglion of *Teleogryllus oceanicus*, namely T103, T301, T504, T701, and T703. All these interneurons were also identified in the present study on *T.commodus* and their respective morphologies and physiologies were confirmed. The role of interneurons T504 and T701 as resetters of the flight rhythm (Robertson 1987) was also confirmed. I have identified 12 further interneurons which are rhythmically active with the flight rhythm in addition to those identified by Robertson (1987). Their respective morphologies are shown in Fig. 3.8. Four of these interneurons have a soma in the mesothoracic ganglion, whereas the others have their somata in the metathoracic ganglion. The interneurons could be grouped into four major physiological classes according to the timing of their bursts and their discharge pattern within a flight cycle.

Class F-I consists of interneurons which are depolarised at the time of the dorsal longitudinal depressor burst. In all cases the entire discharge does not markedly exceed the duration of the dorsal longitudinal burst. Depending on the interneuron the burst may be preceded or followed by IPSPs. Three interneurons fall into this group, namely T309, T510, and T701, and the discharge pattern of T510 is shown as an example in Fig. 3.9 A. This interneuron also shows resetting properties, since intracellular injection of a brief pulse of depolarising current can permanently delay the following flight cycles (Fig. 3.9 A).

Class F-II contains interneurons which are depolarised just prior to the discharge of the metathoracic dorsal longitudinal muscle. The burst generated in these interneurons is usually brief and often followed by a strong IPSP terminating the burst at the time of the dorsal longitudinal discharge. Six interneurons show this burst pattern, i.e. T103, T302, T305, T401, T504, and T508. The burst pattern typical for this class is shown in the recording of interneuron T508 in Fig. 3.9 B. Interneurons T401 and T504 were able to reset the flight rhythm upon intracellular injection of depolarising current.

Fig. 3.8 Drawings showing the morphologies of previously undescribed mesothoracic and metathoracic flight interneurons recorded in the course of this study as seen in wholmount following intracellular staining with Lucifer Yellow. Scale: 200 μ m. Mesothoracic interneurons: T101, T309, T404, and T704. Metathoracic interneurons: T302, T305, T401, T402, T506, T507, T508, and T510. Roman numerals refer to the physiological classes (F-I to F-IV) discussed in the text (see also Fig. 3.9). Previously described flight interneurons (Robertson 1987) were also recorded, but are omitted from this figure.

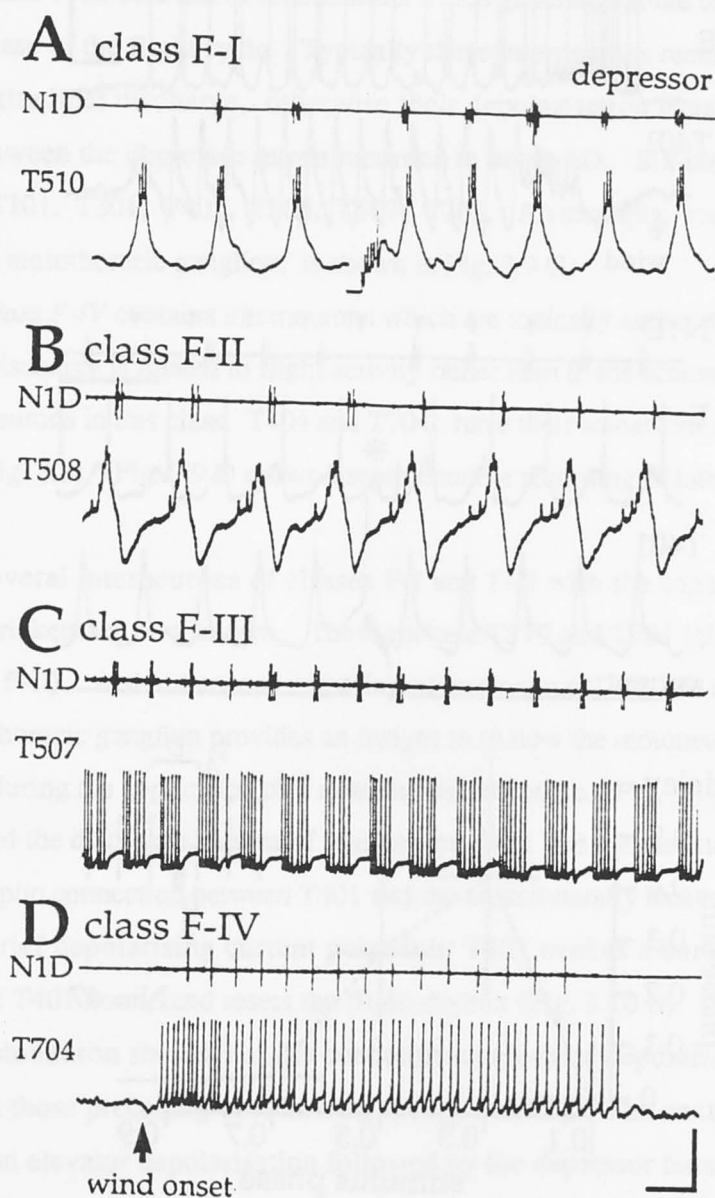


Fig. 3.9 A - D. Recordings from interneurons representative for the various classes discussed in this chapter. The classes were created on the basis of discharge pattern during generation of the flight rhythm (see Fig. 3.8 for the morphologies of the respective interneurons). Upper trace: Metathoracic nerve 1D (N1D) innervating the dorsal longitudinal muscle, a depressor of the hindwing; second trace: intracellular recording of the respective interneuron shown to the left of each recording. **A Class F-I:** Depolarisation occurring at the time of the N1D burst. Interneuron T510 also has resetting properties in that it permanently delays subsequent cycles on current injection. **B Class F-II:** Depolarisation occurring just prior to the N1D discharge. Interneuron T508. **C Class F-III:** Depolarisation lasting during the elevator phase. Interneuron T507. **D Class F-IV:** Tonic discharge during the flight sequence. Interneuron T704. Calibration: Vertical A 15 mV B 10 mV C, D 20 mV; Horizontal A - C 50 ms D 100 ms.

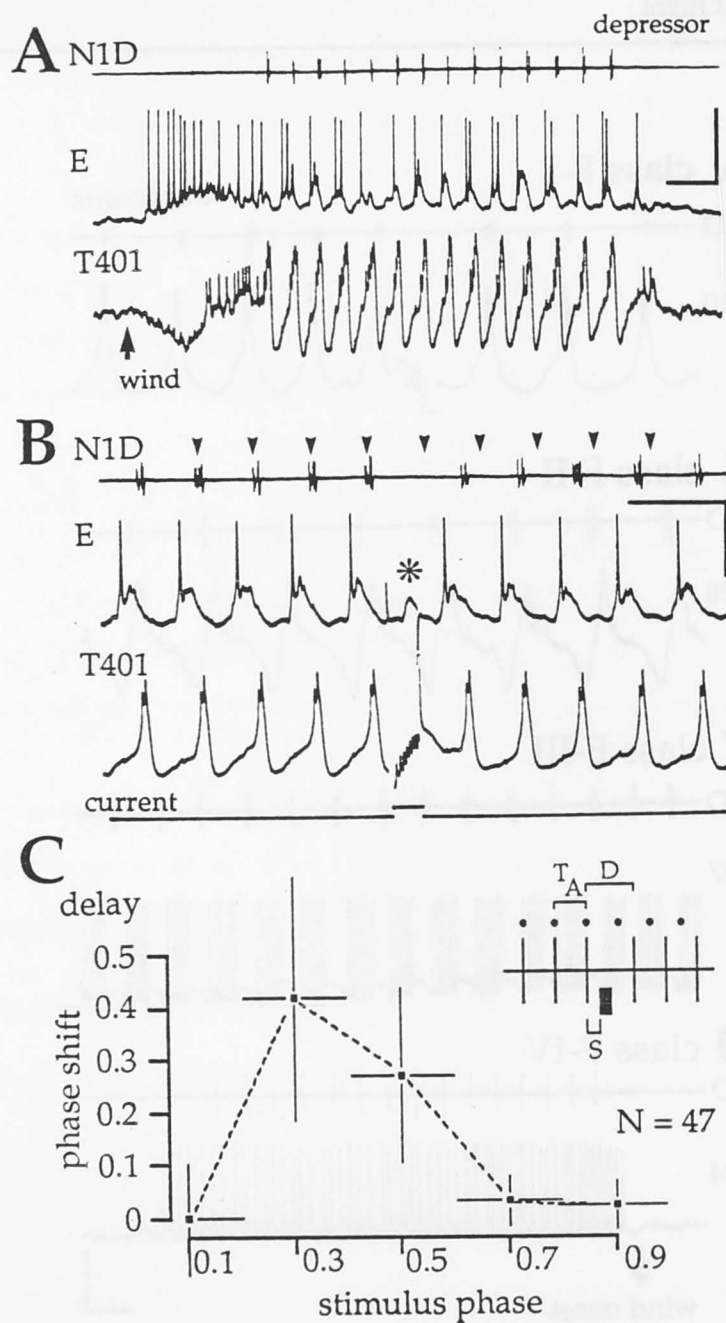


Fig. 3.10 A - C. Interneuron T401 and resetting of the flight rhythm by current injection. **A** Section of a flight sequence showing the activity of interneuron T401 (third trace) and a simultaneously recorded metathoracic elevator motoneuron (E; second trace). There was no connection from the interneuron to the contralaterally located elevator motoneuron. Upper trace: Metathoracic nerve 1D (N1D) innervating the dorsal longitudinal muscle, a depressor of the hindwing. **B** A section of the same recording during which action potentials were elicited in interneuron T401 by passing current intracellularly. The depolarisation in the elevator motoneuron (marked with an asterisk) is weakened and the burst suppressed when current is passed into T401 (see bottom trace for current monitor). The next cycle begins with an elevator depolarisation. The arrowheads (at upper trace) indicate the continuation of the rhythm as it would have been without the current injection. Interneuron T401 was placed in class S-II (a burst just prior to the depressor burst monitored in N1D (Fig. 3.8)), but sometimes bursts extended into the depressor burst as shown in the recording in **B**. **C** Phase response curve summarising the resetting effect of

Class F-III consists of interneurons which generate a tonic depolarisation during the elevator phase of the flight cycle. Typically these interneurons receive IPSPs at the time of dorsal longitudinal discharge, otherwise their depolarisation usually occupies the entire interval between the depressor bursts recorded in nerve 1D. Six interneurons fall into this class, i.e. T101, T301, T402, T506, T507, T703. An example, recorded from the axon of T507 in the mesothoracic ganglion, is shown in Fig. 3.9 C.

Class F-IV contains interneurons which are tonically active during flight activity and where the discharge is related to flight activity rather than to the accompanying wind stimulus. Both interneurons in this class, T404 and T704, have their somata located in the mesothoracic ganglion (Fig. 3.8). Fig. 3.9 D shows a representative recording of interneuron T704.

Several interneurons of classes F-I and F-II with the capacity to reset the flight rhythm in crickets are now known. These include T510 and T701 (class F-I), and T401 and T504 (class F-II). A simultaneous recording of interneuron T401 and an elevator motoneuron in the metathoracic ganglion provides an insight as to how the motoneuronal discharge pattern is changed during the application of a resetting current pulse. Fig. 3.10 A shows a short flight sequence and the discharge pattern of interneuron T401 and the elevator motoneuron. There was no synaptic connection between T401 and the contralaterally located elevator motoneuron. Passing a brief depolarising current pulse into T401 evokes a burst of action potentials, prolongs the T401 burst, and resets the flight rhythm (Fig. 3.10 B). The recording from the elevator motoneuron shows that the concomitant elevator depolarisation is considerably weaker than those preceding or following it (Fig. 3.10 B). The next cycle in the sequence starts with an elevator depolarisation followed by the depressor burst (extracellular trace). This indicates that the next cycle in a flight sequence may only commence when action

interneuron T401. T401 only delayed, but did not advance, the flight cycles. Vertical error bars indicate the standard deviation for the respective phase shifts which are largest when the resetting effects are strongest. Horizontal bars indicate that data within ± 0.1 of the calculated stimulus phases were pooled and so are treated as being of constant length. Inset: calculation of stimulus phase and phase shift. Both variables were normalised by dividing by the mean duration of the two cycles (shown in the inset as the one cycle T_A) immediately preceding the cycle in which the stimulus (black rectangle) was presented (D). The time of presentation of the stimulus (S) was measured from the beginning of the preceding burst in nerve 1D to the beginning of the stimulus (S). To calculate a possible change in rhythm, T_A was subtracted from D. This procedure resulted in positive values for the phase shift, when there was a delay of the next cycle, and negative values for an advance. Calibration: Vertical A, B 20 mV; Horizontal A, B 100 ms.

potentials in the interneuron T401 have ceased. A phase response curve obtained for this interneuron shows that current injection delays the next flight cycle only when the interneuron is stimulated during the first half of a cycle (Fig. 3.10 C). Stimulation later in a period does not have any effect on the flight rhythm, presumably because the bursts in other interneurons (e.g. class F-I, Fig. 3.9 A), can no longer be suppressed.

Another interneuron with a potentially very important role in the generation of the flight rhythm is interneuron T404 (Fig. 3.11). In the locust flight system, passage of current into interneurons with the T404 type morphology elicits a train of action potentials and

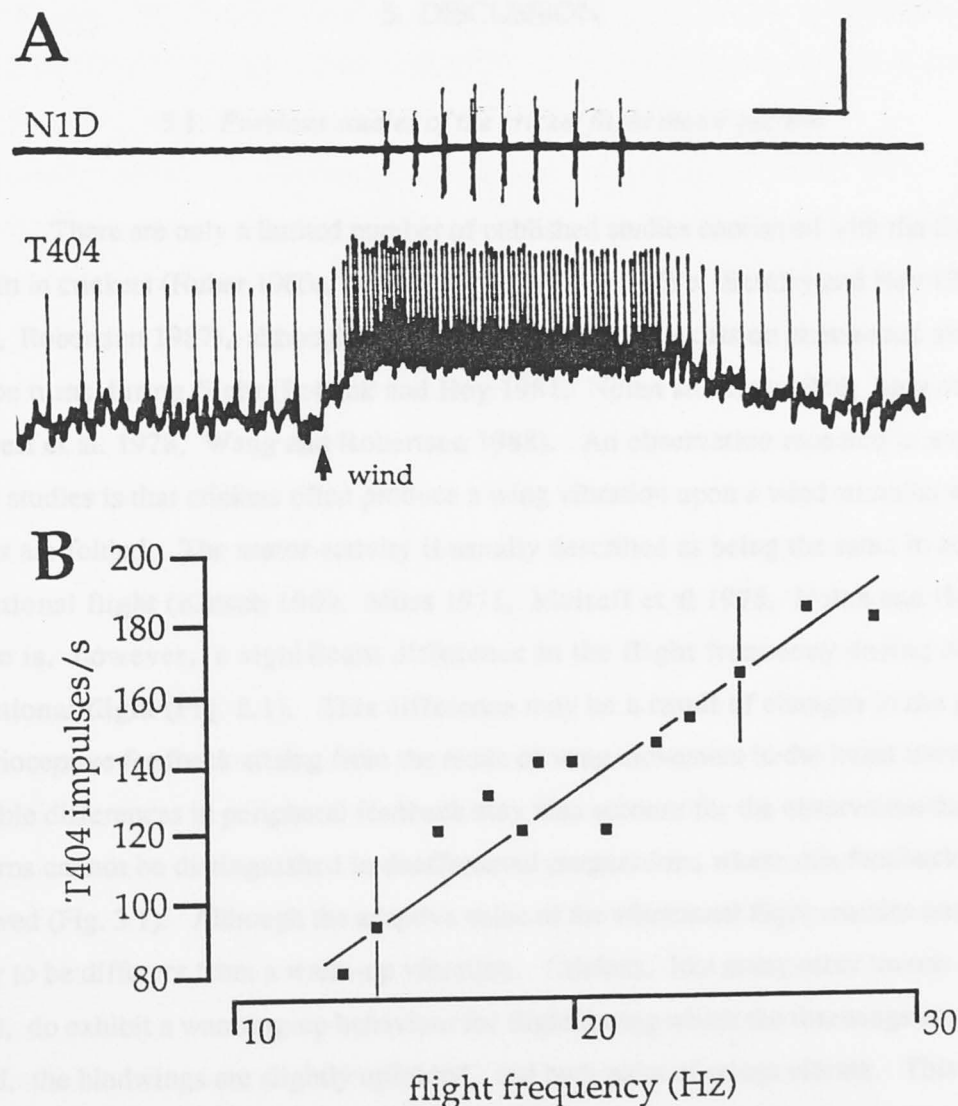


Fig. 3.11 A, B. Physiological characterisation of interneuron T404 (see Fig. 3.8 for morphology of this interneuron). **A** Interneuron T404 is tonically active during a flight sequence. **B** Plotting the T404 discharge against the flight frequency reveals a linear relationship between these two parameters. Calibration: Vertical A 10 mV; Horizontal A 150 ms.

initiates the flight rhythm (Pearson et al. 1985). In the cricket it was not possible, despite attempts in several preparations, to initiate the flight rhythm by passing DC current into the T404. However, the current needed to elicit flight may be quite large, as is the case in the locust (Pearson et al. 1985) and the LY filled electrodes used here limited the amount of current that could be passed. The T404 discharges tonically during the expression of the flight rhythm (Fig. 3.11 A) and its discharge rate is linearly correlated with the flight frequency (Fig. 3.11 B).

5. DISCUSSION

5.1. *Previous studies of the cricket flight motor pattern*

There are only a limited number of published studies concerned with the flight motor pattern in crickets (Huber 1960a, Kutsch 1969, Bentley 1969b, Bentley and Hoy 1970, Möss 1971, Robertson 1987), although a further group of studies exists on phonotactic steering and escape turns during flight (Pollack and Hoy 1981, Nolen and Hoy 1986, May et al. 1988, Moiseff et al. 1978, Wang and Robertson 1988). An observation repeated in a number of these studies is that crickets often produce a wing vibration upon a wind stimulus while their wings are folded. The motor activity is usually described as being the same in active as in vibrational flight (Kutsch 1969, Möss 1971, Moiseff et al 1978, Nolen and Hoy 1986). There is, however, a significant difference in the flight frequency during active and vibrational flight (Fig. 3.1). This difference may be a result of changes in the pattern of proprioceptive feedback arising from the mode of wing movement in the intact animal. Such possible differences in peripheral feedback may also account for the observation that the two patterns cannot be distinguished in deafferented preparations where this feedback has been removed (Fig. 3.1). Although the adaptive value of the vibrational flight remains unclear, it is likely to be different from a warm-up vibration. Crickets, like many other insects (Kammer 1968), do exhibit a warming-up behaviour for flight during which the forewings are distinctly raised, the hindwings are slightly upfolded, and both pairs of wings vibrate. This warm-up behaviour was also often observed here, but could never be evoked in an experimental situation. The failure to distinguish wing-beating and vibrational flight may, however, account for some inconsistencies in the literature on cricket flight, such as apparent variations in flight frequency, and in the forewing to hindwing delay (Bentley 1969b, Kutsch 1969,

Bentley and Hoy 1970, Möss 1971). A significant slowing of the rhythm after deafferentation has now been firmly established (Fig. 3.1; Robertson 1987) and the changes observed correspond to those described for the locust flight system (Wilson 1961, Robertson and Pearson 1982). It appears that the cricket flight motor pattern may vary considerably, since a whole range of burst patterns and cycle durations was observed in this study (Figs. 3.1, 3.2, 3.5).

The description of the discharge of subalar muscle 99 in this study, with a highly variable burst duration, (Fig. 3.5) differs from previous reports which show only one spike per burst (Kutsch 1969, Bentley and Hoy 1970, Möss 1971), as does the intracellularly recorded activity of MN 99 (Fig. 3.5) which does not show the strong phasic component described for MN 99 during locust flight (Hedwig and Pearson 1984). However, the maintained depolarisation of MN 99 was consistently observed in deafferented animals (Figs. 3.5 and 3.7), and sometimes only one action potential was generated by this depolarisation (Fig. 3.5). This variability in the number of action potentials also occurred in intact flying animals (Fig. 3.5 D, E, F). The adaptive value of this discharge pattern is not immediately obvious.

Counting the interneurons reported by Robertson (1987), there are now 17 interneurons described for the cricket flight system. The discharge patterns of these interneurons may be grouped roughly into four classes (Fig. 3.9). The first three classes, containing interneurons with a phasic activity pattern, allow one, in principle, to describe the motoneuronal burst pattern by assuming excitatory and inhibitory interneurons with appropriate discharge properties (Fig. 3.12). Some of these interneurons may be members of the same physiological class and the interneurons recorded in this study allow one to account for such different connections, if one applies the proposal of Pearson and Robertson (1987; see Robertson 1987 for the cricket) that structure may predict function. According to their proposal, interneurons T701 (class F-I), T302, T504, T508 (F-II), and T301 (F-III) would be expected to have excitatory postsynaptic effects, whereas interneurons T309 and T501 (F-I), and T305 and T401 (F-II) should have inhibitory postsynaptic effects (see Fig. 3.8 for morphologies). If these predictions are correct the activity of these interneurons alone can account for the basic burst pattern observed in motoneurons (Fig. 3.12).

As demonstrated, classes F-I and F-II contain resetter interneurons (Figs. 3.9 and 3.10; Robertson 1987). Importantly, the three phasic classes indicate that the short depressor burst might be a crucial event in a flight cycle, since it appears to terminate the otherwise maintained elevator discharge and, due to its limited duration, appears to release the next

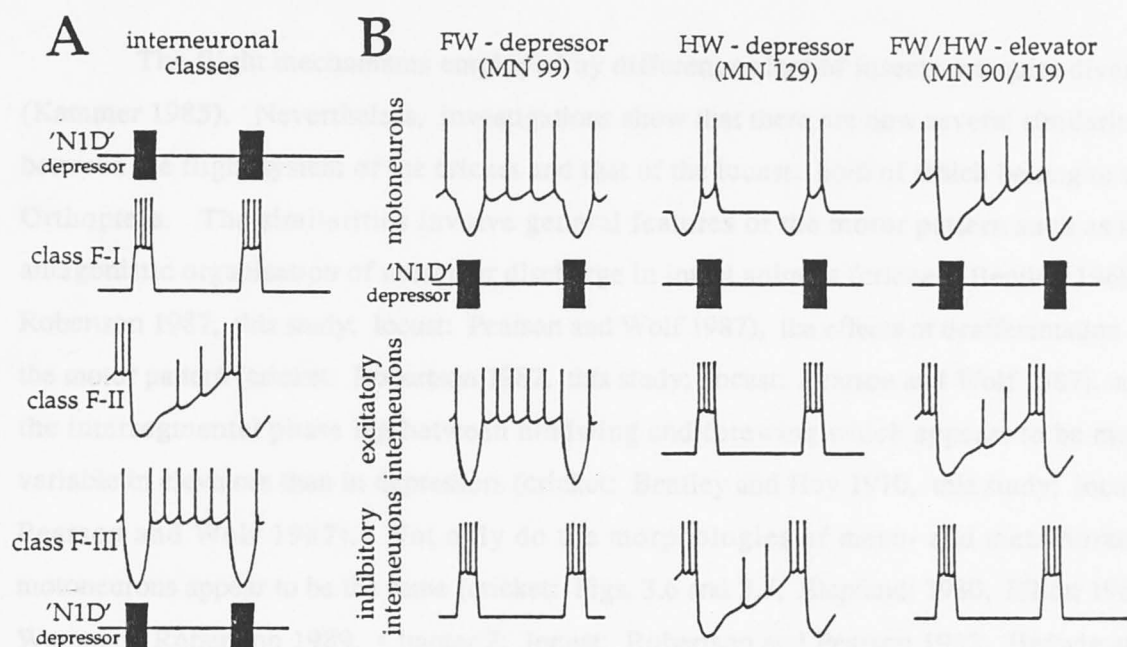


Fig. 3.12 A, B. Interneuronal discharge patterns and their relation to phases of activity in motoneurons. **A** Schematic presentation of the interneuronal burst patterns encountered in this study (see Fig. 3.9). **B** Schematic presentation of the motoneuronal burst patterns described in this study (see Figs. 3.3 - 3.6). Elevator burst patterns do not differ and are therefore treated as one, although a lag may separate their bursts in the mesothoracic and metathoracic ganglia. Below each motoneuronal burst are indicated interneuronal discharge patterns which could produce the observed shape of the motoneuronal burst. In principle only two interneurons are needed for each burst pattern, one class of interneurons with excitatory effects (upper row) and another class with inhibitory effects (lower row).

cycle, starting with an accumulating excitation in elevators. This elevator to depressor coupling (Fig. 3.6; Robertson 1987), which results in asymmetrical cycles, is similar to that described for the flight system of locust (Hedwig and Pearson 1984, Robertson and Pearson 1985). Furthermore, the coupling of synaptic events such as inhibition to excitation (Robertson 1987) and excitation to inhibition (e.g. interneurons T508 (Fig. 3.9) and T401 (Fig. 3.10)) always occurs at the time of the depressor burst. This suggests that there is a crucial event in cycle generation at that time and makes a central coupling of interneuronal classes F-I and F-II, which also contain all resetter interneurons reported to date, very likely.

5.2. Comparison with the locust flight system

The flight mechanisms employed by different orders of insects are quite diverse (Kammer 1985). Nevertheless, investigations show that there are now several similarities between the flight system of the cricket and that of the locust, both of which belong to the Orthoptera. The similarities involve general features of the motor pattern such as the antagonistic organisation of muscular discharge in intact animals (cricket: Bentley 1969b, Robertson 1987, this study; locust: Pearson and Wolf 1987), the effects of deafferentation on the motor pattern (cricket: Robertson 1987, this study; locust: Pearson and Wolf 1987), and the intersegmental phase lag between hindwing and forewing which appears to be more variable in elevators than in depressors (cricket: Bentley and Hoy 1970, this study; locust: Pearson and Wolf 1987). Not only do the morphologies of meso- and metathoracic motoneurons appear to be the same (cricket: Figs. 3.6 and 3.7, Elepfandt 1980, Elliott 1983, Wang and Robertson 1989, Chapter 2; locust: Robertson and Pearson 1982, Hedwig and Pearson 1984), but the burst patterns of meso- and metathoracic motoneurons are also very similar, with the exception of the cricket mesothoracic subalar motoneuron which appears to be specific to the cricket (cricket: Figs. 3.3 - 3.7; locust: Hedwig and Pearson 1984). While mesothoracic and metathoracic elevator motoneurons appear to receive very similar patterns of synaptic input in cricket and locust, depressor motoneurons show interspecific differences (cricket: Figs. 3.6 and 3.7; locust: Hedwig and Pearson 1984). The elevator to depressor coupling which is observed in both the cricket (Robertson 1987, this study) and the locust (Hedwig and Pearson 1984, Robertson and Pearson 1985) indicates similar mechanisms of motor pattern generation. To date, only spiking interneurons have been described for the flight system of the cricket and the locust, although some of these may also use graded synaptic transmission (Robertson and Reye 1988). This contrasts with the walking system, at least in the locust, where non-spiking interneurons play a major role in neuronal integration of sensory information (Burrows and Siegler 1978).

Many of the interneurons recorded during this study share similarities in morphology and physiology with interneurons recorded in the locust. Specific resemblances include (cricket/locust): T103/401, T309/302, T401/401, T404/404, T504/504 (Fig. 3.13). Importantly, among these pairings are interneurons which are capable of permanently influencing the rhythm such as T504 (Fig. 3.13; Robertson 1987; confirmed in this study) and T401 (Figs. 3.10 and 3.13). Furthermore, interneuron T404 appears to possess the same

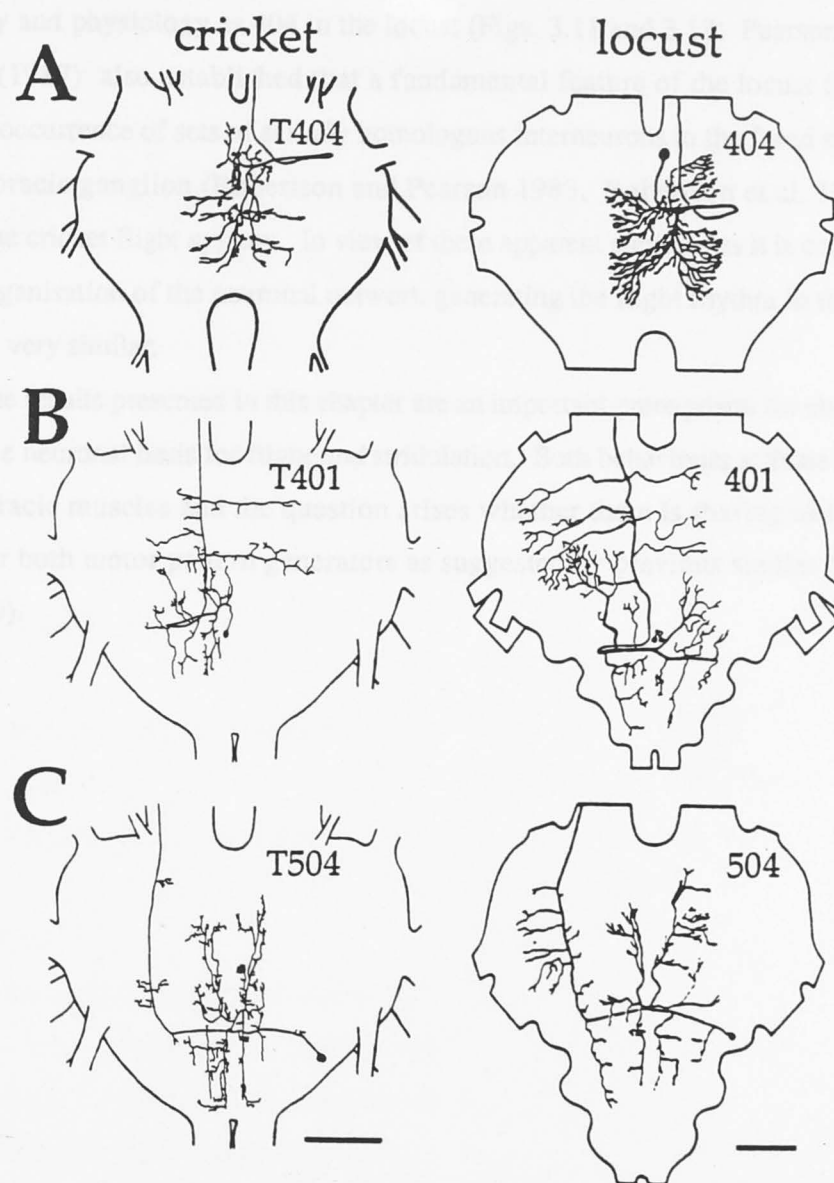


Fig. 3.13 A - C. A comparison of interneurons patterning flight activity in the cricket (left column) and the locust (right column; interneuronal drawings for the locust were taken from Pearson et al. (1985; 404) and Robertson and Pearson (1983; 401, 504). **A** Interneurons T404 and 404 show the same major branching pattern in the mesothoracic ganglion and they are both tonically active with the flight rhythm. **B** Interneurons T401 and 401 show a characteristic loop in the metathoracic ganglion, they are active in the same phase of the flight cycle, and they may reset the flight rhythm permanently. **C** Interneurons T504 and 504 are located posteriorly in the metathoracic ganglion and show similar branching patterns. Both interneurons are capable of resetting the flight rhythm. Scale 200 μ m.

morphology and physiology as 404 in the locust (Figs. 3.11 and 3.13; Pearson et al. 1985). Robertson (1987) also established that a fundamental feature of the locust flight system, namely the occurrence of sets of serially homologous interneurons in the fused neuromeres of the metathoracic ganglion (Robertson and Pearson 1983, Robertson et al. 1982), is also present in the cricket flight system. In view of these apparent similarities it is conceivable that the basic organisation of the neuronal network generating the flight rhythm in the cricket and the locust is very similar.

The results presented in this chapter are an important prerequisite for chapter 4 which compares the neuronal basis for flight *and* stridulation. Both behaviours activate the same sets of mesothoracic muscles and the question arises whether there is sharing of interneuronal circuitry for both motor pattern generators as suggested by previous studies (Huber 1962, Kutsch 1969).

CHAPTER 4

NEURONAL CONTROL OF THE FOREWINGS
IN TWO DIFFERENT BEHAVIOURS:
STRIDULATION AND FLIGHT IN THE CRICKET,
TELEOGRYLLUS COMMODUS

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1. SUMMARY

The forewing of the cricket is activated during the performance of two different behaviours, flight and stridulation. Intracellular recording and staining techniques were employed to determine the neuronal basis for these two behaviours and how they are interrelated.

(1) Bifunctional mesothoracic motoneurons (MN 90, MN 99) are activated with different patterns of synaptic input during flight and stridulation (Fig. 4.2).

(2) Metathoracic flight motoneurons (MN 112, MN 119, MN 129) are only activated during flight, but do not receive phasic excitatory input during stridulation (Figs. 4.3 and 4.6).

(3) Two separate interneuronal pools, which include interneurons with resetter properties, appear to exist, one activated during flight, the other one activated during stridulation (Figs. 4.4 and 4.5). The interactions between these two pools are inhibitory. Both motor patterns may be switched rapidly (less than 0.5 s; Fig. 4.6).

(4) It is possible to elicit both behaviours at the same time and then no coupling is observed between them (Figs. 4.7 to 4.9).

These observations suggest that there are two distinct neural networks which control flight and stridulation.

2. INTRODUCTION

An animal may use the same set of muscles to activate one limb or appendage in different behaviours such as scratching and walking (cat) or stridulation and flight (cricket). The question of how the neuronal control of behaviours is organised under such circumstances has generated interest for many years (Sherrington 1906). Recent approaches using electromyographic (EMG) analysis have shown that there are strong similarities between the motor patterns used in the generation of different behaviours in both vertebrates and invertebrates (table 4.1) and that there is some sharing of interneuronal circuitry (see Heitler (1985) for an exception).

Table 4.1: Descriptions of different motor patterns involving the same limb (appendage)

<i>animal</i>	<i>motor patterns</i>	<i>limb (appendage)</i>	<i>reference</i>
<i>INVERTEBRATES</i>			
crab	scaphognathite beating	scaphognathite	Simmers and Bush 1983
crayfish	swimmeret beating	swimmeret	Heitler 1985
lobster	walking	leg	Ayers and Davis 1977, Ayers and Clarac 1978
cockroach	walking, righting, grooming	leg	Sherman et al. 1977, Reingold and Camhi 1977, Zill 1986
cricket	stridulation, flight	forewing	Huber 1962, Kutsch 1969
locust	jumping, swimming, kicking	leg	Pflüger and Burrows 1978
moth	flight, warm-up	wings	Kammer 1968
<i>VERTEBRATES</i>			
cat	walking and scratching	leg	Berkinblit et al. 1978
	walking and paw-shake	leg	Carter and Smith 1986
chicken	walking, hatching	leg	Bekoff 1986
turtle	three scratch patterns	leg	Robertson et al. 1985

A sharing of the neuronal circuitry controlling the bifunctional muscles which activate the leg during stridulation, and the wing during flight, in grasshoppers has been suggested by Elsner (1974, 1983). On the other hand, Ramirez and Pearson (1988), using intracellular recording techniques, recently demonstrated that the bifunctional muscles for

walking and flight in the locust (Wilson 1962) are driven by largely separate interneuronal networks each generating one of the motor patterns. The cricket not only employs the same sets of muscles, but also the same appendage (i.e. the forewing) in two quite different rhythmical behaviours, namely stridulation and flight (Fig. 4.1). This makes the cricket ideally suited to an investigation concerned with the organisation of neuronal circuits involved in different motor patterns.

During stridulation the cricket raises the forewings and moves them rhythmically against one another (Fig. 4.1 A). Sound is produced only during the closing movement and the alternating opening and closing movements during stridulation generate syllables as the scraper rubs against the file on the underside of the upper right wing (Huber 1960a, Kutsch 1969, Koch et al. 1988). The species-specific sound pattern of the *Teleogryllus commodus* calling song contains two different alternating rhythms (Bentley and Hoy 1972; Chapter 2): the chirp, which consists of syllables repeated at ~20 Hz; and the trill, which consists of syllables repeated at ~35 Hz. The principal muscles involved in producing both chirp and trill in stridulation are muscles 98 (2nd basalar) and 99 (subalar) which open the wings, and muscles 89 (promotor coxae) and 90 (remotor coxae) which close them (Kutsch 1969). The morphologies of the motoneurons innervating these muscles in the mesothoracic ganglion have been described previously (Elepfandt 1980, Elliott 1983, Wang and Robertson 1989, Chapter 2) as has the role of other muscles active during stridulation (for details see Kutsch 1969). In contrast to stridulation, during flight the wings (including the hindwings which are not shown in Fig. 4.1) are unfolded and beat regularly up and down (Fig. 4.1 B). The principal muscles active during flight are the same ones active during stridulation although their action differs: muscles 98 and 99 depress the wings, while muscles 89 and 90 elevate them. These muscles are also involved in leg movements during walking which makes them trifunctional (Wilson 1962, Huber 1963, Ramirez and Pearson 1988, Wang and Robertson 1989).

The motor patterns underlying both the stridulatory and flight behaviours are well described (Bentley 1969a, 1969b, Kutsch 1969, Möss 1971, Bentley and Hoy 1972) and both motor rhythms may be elicited in preparations which allow intracellular recording from central neurons (Bentley 1969a, 1969b, Robertson 1987, Chapters 2 and 3). The present study demonstrates that motoneurons receive different patterns of synaptic input during the expression of the stridulatory and flight rhythm; that probably two separate pools of interneurons exist for each rhythm; and that both motor patterns may be elicited

simultaneously. These findings suggest that each motor pattern is generated by a separate network, and that these networks converge on the mesothoracic musculature in order to accomplish the appropriate forewing movement.

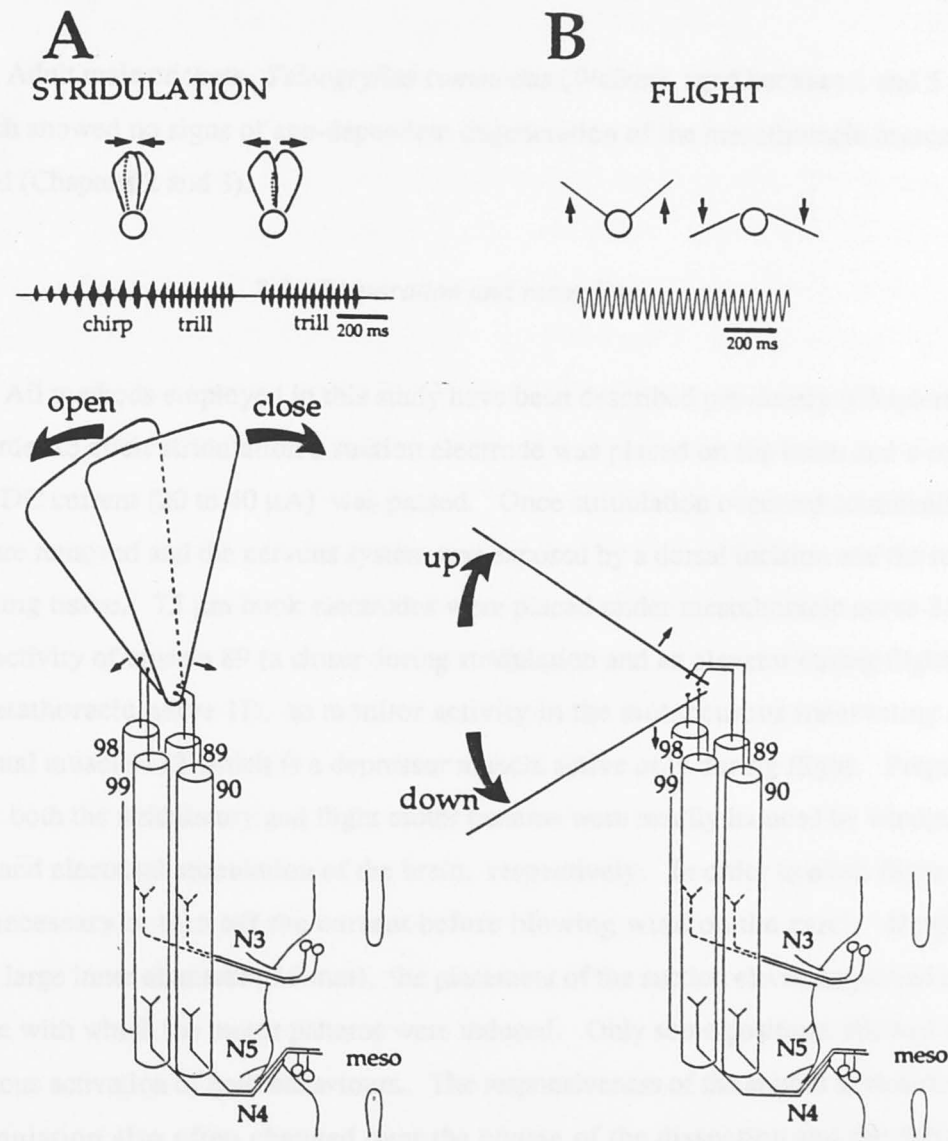


Fig. 4.1 A, B. Schematic presentation of the functional anatomy of the mesothoracic segment of a cricket showing the movement patterns of the forewings during stridulation and flight. **A** The opening and closing movements during stridulation generate a species-specific sound pattern which in *Teleogryllus commodus* contains two different alternating rhythms, the chirp and the trill. During stridulation the forewings are raised and moved rhythmically against one another. The main muscles accomplishing this movement, and their action, are indicated for the left side of the mesothoracic segment. Muscles 98 and 99 open the wings, whereas muscles 89 and 90 close them. The projections of the motoneurons innervating these muscles in the mesothoracic ganglion are indicated. **B** During flight, the wings (including the hindwings which are not shown here) are unfolded and beat regularly up and down. The major muscles active during flight are the same as during stridulation although their action differs. Muscles 98 and 99 depress the wings and muscles 89 and 90 elevate them.

3. MATERIALS AND METHODS

3.1. Animals

Adult male crickets, *Teleogryllus commodus* (Walker) aged between 1 and 5 weeks, and which showed no signs of age-dependent degeneration of the mesothoracic musculature, were used (Chapters 2 and 3).

3.2. Preparation and recording

All methods employed in this study have been described previously (Chapters 2 and 3). In order to elicit stridulation a suction electrode was placed on the brain and a constant negative DC current (20 to 40 μ A) was passed. Once stridulation occurred continually, the wings were removed and the nervous system was exposed by a dorsal incision and the removal of overlying tissue. 75 μ m hook electrodes were placed under mesothoracic nerve 3A3, to monitor activity of muscle 89 (a closer during stridulation and an elevator during flight), and under metathoracic nerve 1D, to monitor activity in the motoneurons innervating dorsal longitudinal muscle 112 which is a depressor muscle active only during flight. Prepared in this way, both the stridulatory and flight motor patterns were readily induced by windpuffs on the cerci and electrical stimulation of the brain, respectively. In order to elicit flight it was usually necessary to turn off the current before blowing wind on the cerci. Despite its relatively large inner diameter (0.5 mm), the placement of the suction electrode proved critical to the ease with which the motor patterns were induced. Only some positions allowed for the simultaneous activation of both behaviours. The responsiveness of the animal to electrical and wind stimulation also often changed over the course of the dissection and the following experiment (1 - 2 h).

All other details of the preparation, the recording electrodes and techniques, are as previously described (Chapters 2 and 3). Interneurons are identified according to the scheme proposed by Robertson (1987), the nomenclature of muscles follows Kutsch (1969), and that of nerves follows Kutsch and Huber (1970).

4. RESULTS

4.1. *Synaptic input to motoneurons*

4.1.1. *Mesothoracic motoneurons, MN 90, MN 99*

Rhythmical activity in MN 90 during stridulation consists of an IPSP followed by a strong excitation generating a burst of action potentials, which then slowly decays (Fig. 4.2 A). The burst occurs in phase with activity in closer muscle 89. During flight, however, there is a more gradual depolarisation which peaks in a short burst of action potentials just prior to the burst in the dorsal longitudinal muscle (recorded in metathoracic nerve 1D), a depressor of the hindwing (Fig. 4.2 B). IPSPs may occur during the repolarisation phase of this elevator burst (Fig. 4.2 B; Chapter 3). The pattern of excitation and inhibition in MN 90 therefore differs during stridulation and flight. The bursts in depressor MN 99 occur in antiphase to those in MN 90 during stridulation as well as during flight. During stridulation, a phasic depolarisation generates an action potential doublet which is present in both the chirp and trill (Fig. 4.2 C, only the chirp section is shown here; Chapter 2), but which is never observed during flight activity. During flight MN 99 shows a more tonic depolarisation supporting action potentials, which is followed by an IPSP approximately coincident with metathoracic dorsal longitudinal muscle activity (Fig. 4.2 D). While MN 99 receives regular IPSPs at the time of metathoracic depressor activity during flight, there are usually no IPSPs observed in this motoneuron during stridulation.

4.1.2. *Metathoracic motoneurons, MN 112, MN 129, MN 119*

The metathoracic dorsal longitudinal and subalar muscles are innervated by MN 112 and MN 129, respectively. During stridulation, these motoneurons usually receive no phasic synaptic input (Fig. 4.3 A, C). Occasionally IPSPs phase coupled to the stridulatory motor pattern are observed (dashed lines in Fig. 4.3 C). During flight, both motoneurons receive a brief excitation which generates 1 - 3 action potentials (Fig. 4.3 B, D). Elevator motoneuron 119 exhibits the same patterns of synaptic input during stridulation as MN 112 and MN 129 and also shows strong rhythmical activity during flight (cf. Fig. 4.6 A, B).

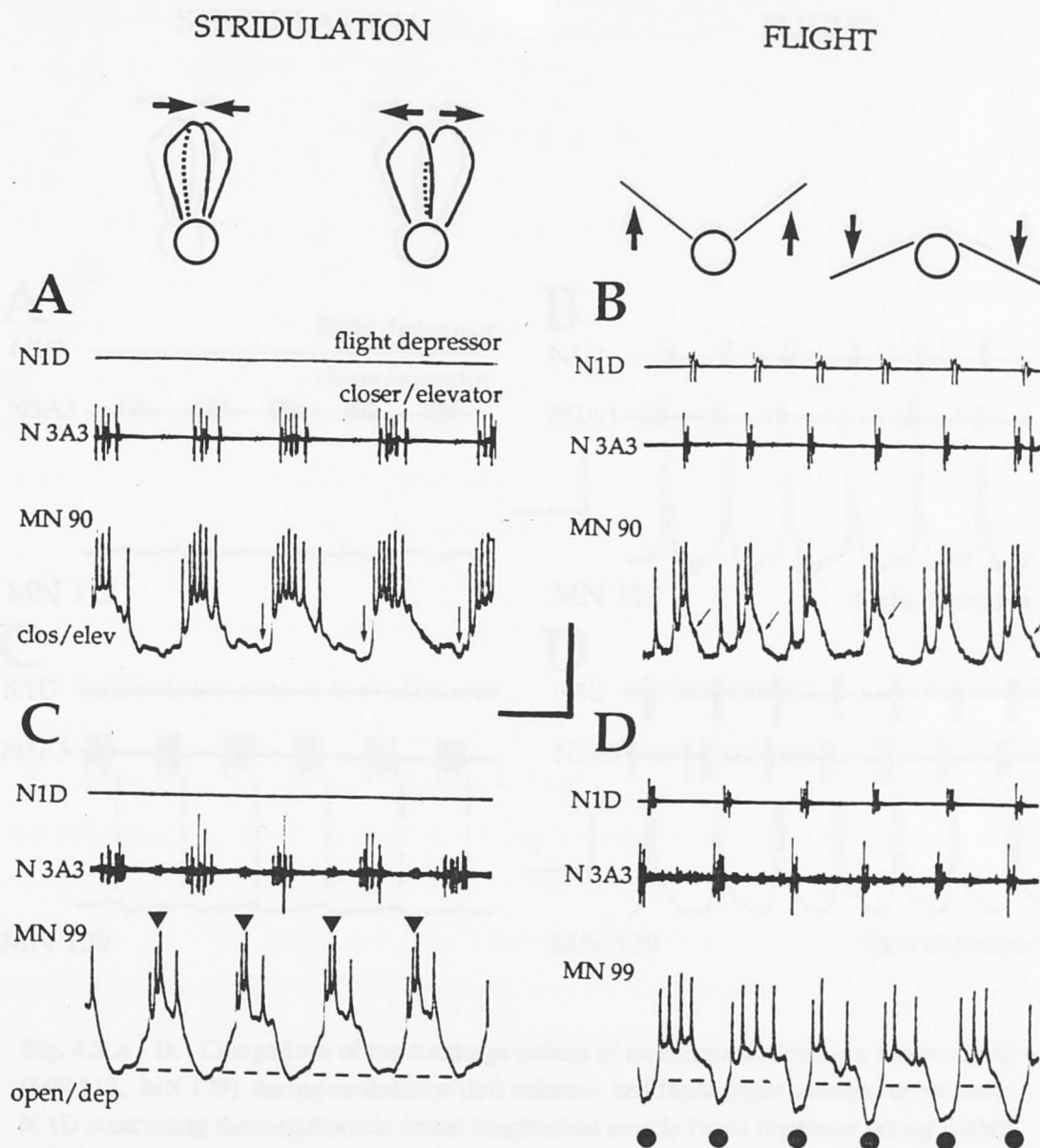


Fig. 4.2 A - D. Comparison of the discharge pattern of bifunctional mesothoracic motoneurons during stridulation (left column) and flight (right column; upper trace: N1D innervating the metathoracic dorsal longitudinal muscle (wing depressor during flight); middle trace: nerve 3A3 innervating elevator (closer) muscle 89 of the forewing; lower trace: intracellular recordings of mesothoracic motoneurons 90 and 99. IPSPs are marked with an arrow. **A, B** During stridulation (**A**) MN 90 exhibits a fast rising depolarisation which is preceded by an IPSP, whereas during flight (**B**) the depolarisation is more gradual and followed by IPSPs. **C, D** During stridulation (**C**) MN 99 shows a phasic depolarisation which results in a characteristic action potential doublet (arrowhead), whereas during flight (**D**) the depolarisation is more tonic than phasic and interrupted by an IPSP (filled circle) at the time of N1D discharge. Dashed line indicates resting potential. Calibration: Vertical A - D 20 mV; Horizontal A - D 50 ms.

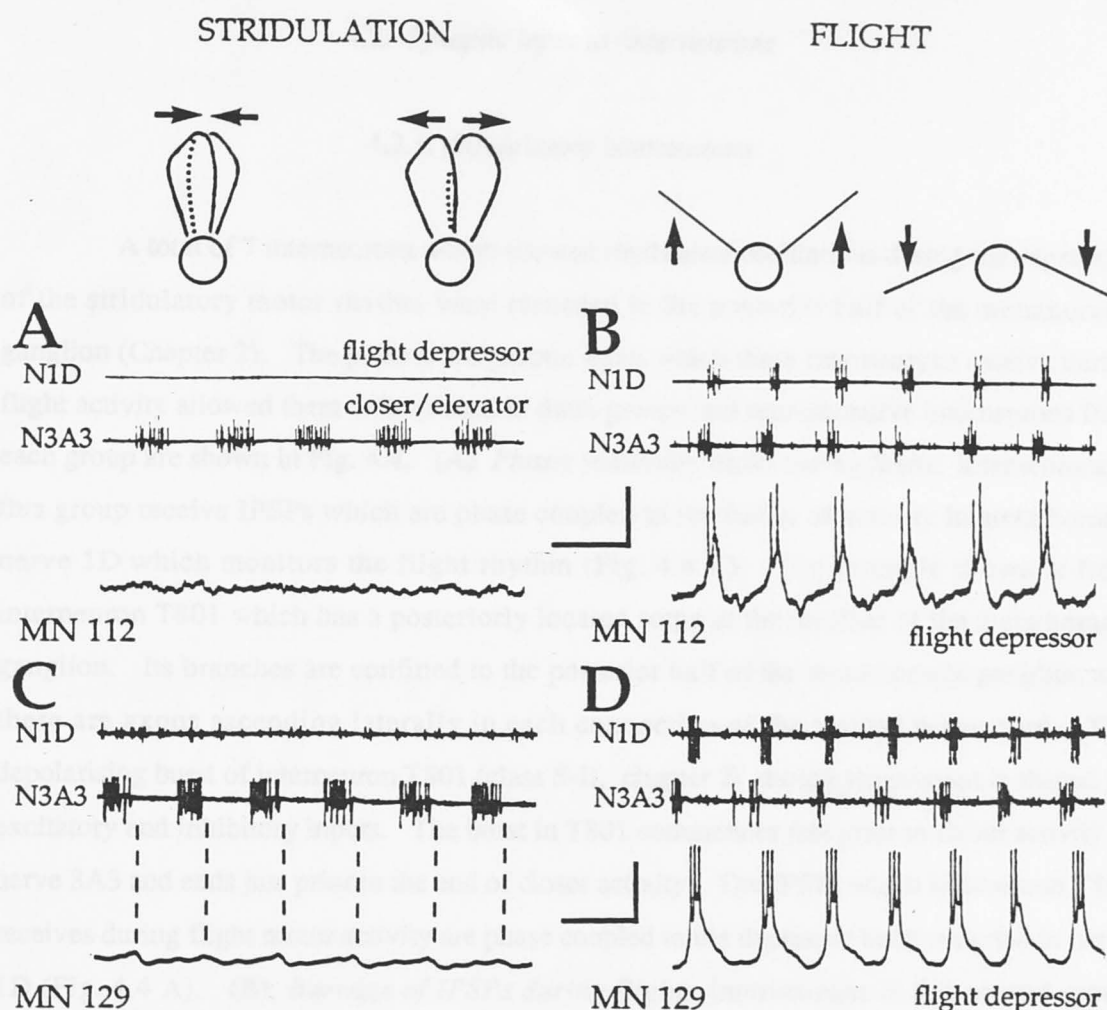


Fig. 4.3 A - D. Comparison of the discharge pattern of metathoracic depressor motoneurons (MN 112, MN 129) during stridulation (left column) and flight (right column; upper trace: N 1D innervating the metathoracic dorsal longitudinal muscle (wing depressor during flight); middle trace: nerve 3A3 innervating elevator (closer) muscle 89 of the forewing; lower trace: intracellular recordings of metathoracic motoneurons 112 and 129. **A, B** MN 112 is rhythmically depolarised in phase with the flight rhythm (**B**), but during stridulation receives no synaptic input which is different from a quiescent preparation (**A**). **C, D** The same is observed for subalar MN 129 (**C** stridulation; **D** flight). In some preparations phase coupled IPSPs (see dashed lines in **C**) are observed during stridulation. Calibration: Vertical **A - D** 20 mV; Horizontal **A - D** 50 ms.

These different patterns of synaptic input during stridulation and flight suggest that the bifunctional mesothoracic motoneurons might be driven by largely different pools of premotor interneurons during the expression of the two behaviours, and further, that the premotor flight interneurons activating the motoneurons of the hindwing musculature are silent during stridulation.

4.2. Synaptic input to interneurons

4.2.1. Stridulatory interneurons

A total of 7 interneurons which showed rhythmical oscillations during the expression of the stridulatory motor rhythm were recorded in the posterior half of the metathoracic ganglion (Chapter 2). The pattern of synaptic input which these interneurons receive during flight activity allowed them to be placed in three groups and representative interneurons from each group are shown in Fig. 4.4. (A) *Phasic inhibitory input during flight*: interneurons in this group receive IPSPs which are phase coupled to the bursts of activity in metathoracic nerve 1D which monitors the flight rhythm (Fig. 4.4 A). The example shown is from interneuron T801 which has a posteriorly located soma at the midline of the metathoracic ganglion. Its branches are confined to the posterior half of the metathoracic ganglion and there are axons ascending laterally in each connective of the ventral nerve cord. The depolarising burst of interneuron T801 (class S-II, chapter 2) during stridulation is shaped by excitatory and inhibitory inputs. The burst in T801 commences just prior to closer activity in nerve 3A3 and ends just prior to the end of closer activity. The IPSPs which interneuron T801 receives during flight motor activity are phase coupled to the depressor burst recorded in nerve 1D (Fig. 4.4 A). (B) *Barrage of IPSPs during flight*: interneurons in this second group receive a barrage of IPSPs during the expression of the flight motor pattern (Fig. 4.4 B). The soma of interneuron T306 is located posteriolaterally in the metathoracic ganglion, the major branches are restricted to the midline, and the axon descends laterally in the contralateral connective of the abdominal nerve cord. The burst pattern of interneuron T306 (class S-I, chapter 2) during stridulation consists of a brief depolarisation in antiphase to the closer discharge in nerve 3A3 and this depolarisation is terminated by IPSPs at the time of the closer burst. During the expression of flight motor activity T306 receives a continuous barrage of IPSPs which do not appear to be phase coupled to a flight cycle. (C) *No change in synaptic input during flight as compared with a quiescent animal*: the third group of interneurons shows no changes in the pattern of synaptic input as compared with a quiescent animal (Fig. 4.4 C). The soma of interneuron T308 is located posteriolaterally in the metathoracic ganglion where the descending connectives exit. There are two dense areas of arborisations confined to the midline and the posterior half of the metathoracic ganglion. The axon descends contralaterally in the abdominal nerve cord. During stridulation interneuron T308

stridulatory interneurons

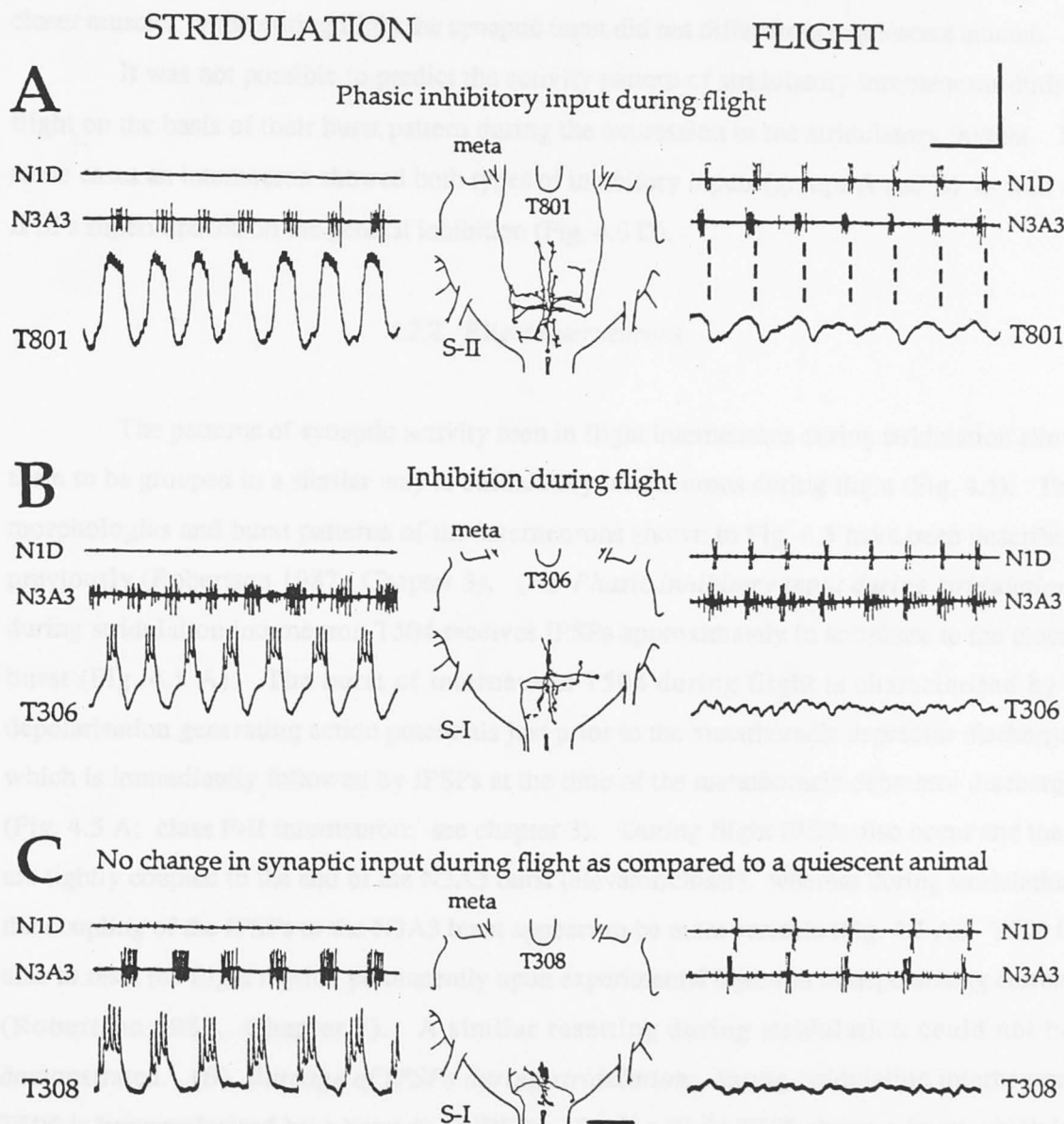


Fig. 4.4. A - C. Stridulatory interneurons and types of synaptic input during flight. Left column: activity during stridulation; center column: interneuron structure drawn from wholemount following intracellular staining with Lucifer Yellow; right column: activity during flight. Each recording shows extracellularly recorded activity of nerve 1D (innervating the metathoracic dorsal longitudinal muscle, upper trace), extracellularly recorded activity of nerve 3A3 (innervating mesothoracic muscle 89, middle trace), and the respective interneuron (bottom trace). **A** Interneuron T801 has two ascending axons. During stridulation T801 shows a depolarising burst approximately at closer activity (N3A3), whereas during flight there are IPSPs phase coupled to the N1D burst (indicated by the dashed lines). **B** Interneuron T306 has an axon descending in the abdominal connective. During stridulation T306 bursts are in antiphase to closer activity (N3A3), but during flight there is a barrage of IPSPs without any phasic coupling to the flight rhythm. **C** Interneuron T308 has arborisations confined to the posterior part of the metathoracic ganglion and an axon descending in the abdominal connective. During stridulation it bursts in antiphase to the closer activity, but during flight there is no change in synaptic input as compared with a quiescent animal. Scale 200 μ m. Calibration: Vertical A, C 20 mV B 25 mV; Horizontal A - C 100 ms.

(class S-I, chapter 2) showed a burst in antiphase to the extracellularly recorded activity in a closer muscle, while during flight the synaptic input did not differ from a quiescent animal.

It was not possible to predict the activity pattern of stridulatory interneurons during flight on the basis of their burst pattern during the expression of the stridulatory rhythm. In some cases an interneuron showed both types of inhibitory inputs (groups A and B) as well as IPSPs superimposed on the general inhibition (Fig. 4.6 D).

4.2.2. *Flight interneurons*

The patterns of synaptic activity seen in flight interneurons during stridulation allow them to be grouped in a similar way to stridulatory interneurons during flight (Fig. 4.5). The morphologies and burst patterns of the interneurons shown in Fig. 4.5 have been described previously (Robertson 1987, Chapter 3). (A) *Phasic inhibitory input during stridulation*: during stridulation interneuron T504 receives IPSPs approximately in antiphase to the closer burst (Fig. 4.5 A). The burst of interneuron T504 during flight is characterised by a depolarisation generating action potentials just prior to the metathoracic depressor discharge, which is immediately followed by IPSPs at the time of the metathoracic depressor discharge (Fig. 4.5 A; class F-II interneuron: see chapter 3). During flight IPSPs also occur and they are tightly coupled to the end of the N3A3 burst (elevator/closer), whereas during stridulation the coupling of the IPSPs to the N3A3 burst appears to be more variable (Fig. 4.5 A). T504 is able to reset the flight rhythm permanently upon experimental injection of depolarising current (Robertson 1987, Chapter 3). A similar resetting during stridulation could not be demonstrated. (B) *Barrage of IPSPs during stridulation*: during stridulation interneuron T506 is hyperpolarised by a barrage of IPSPs. During flight T506 shows a burst which is shaped by strong excitatory inputs, as well as inhibitory inputs in antiphase with the metathoracic depressor activity (Fig. 4.5 B; class F-III interneuron: see chapter 3). (C) *No change in synaptic input during stridulation as compared with a quiescent animal*: interneuron T101 is a spiking local interneuron in the mesothoracic ganglion. During stridulation this interneuron shows no change in synaptic input compared with its activity in a quiescent animal. During flight, T101 shows a burst in antiphase to the depressor discharge (Fig. 4.5 C, class F-III interneuron, see chapter 3).

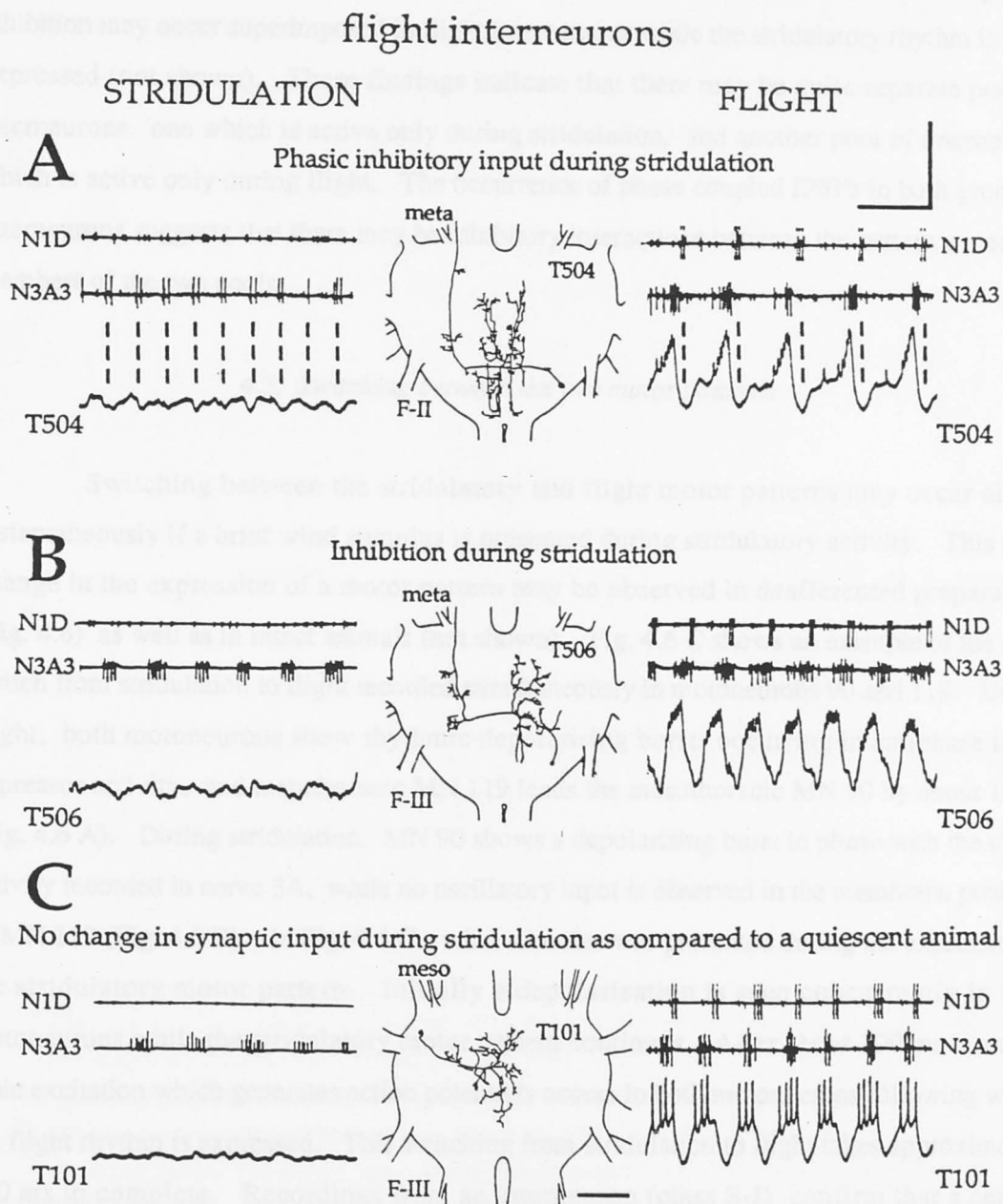


Fig. 4.5 A - C. Flight interneurons and types of synaptic input during stridulation. Columns and recording traces as in Fig. 4.4. **A** During stridulation interneuron T504 receives IPSPs (dashed lines) which are approximately in phase with the closer burst of the stridulatory rhythm, whereas during flight there is a strong depolarisation followed by an IPSP (dashed line) just prior to the burst in nerve 1D. **B** During stridulation interneuron T506 receives a barrage of IPSPs which are not phasically coupled to the stridulatory rhythm. During flight there is a depolarising burst in antiphase to the depressor discharge. **C** During stridulation local mesothoracic interneuron T101 shows no change in synaptic input as compared with a quiescent animal. However, during flight T101 bursts in antiphase to the depressor activity. Calibration: Vertical A 20 mV B, C 15 mV; Horizontal A - C 100 ms.

As already described for stridulatory interneurons (Fig. 4.4), IPSPs and a general inhibition may occur superimposed in flight interneurons while the stridulatory rhythm is being expressed (not shown). These findings indicate that there may be quite separate pools of interneurons, one which is active only during stridulation, and another pool of interneurons which is active only during flight. The occurrence of phase coupled IPSPs in both groups of interneurons suggests that there may be inhibitory interactions between the pattern generating members of the two pools.

4.3. *Switching between the two motor patterns*

Switching between the stridulatory and flight motor patterns may occur almost instantaneously if a brief wind stimulus is presented during stridulatory activity. This rapid change in the expression of a motor pattern may be observed in deafferented preparations (Fig. 4.6) as well as in intact animals (not shown). Fig. 4.6 C shows an example of the rapid switch from stridulation to flight recorded simultaneously in motoneurons 90 and 119. During flight, both motoneurons show rhythmic depolarising bursts occurring in antiphase to the depressor activity, and metathoracic MN 119 leads the mesothoracic MN 90 by about 10 ms (Fig. 4.6 A). During stridulation, MN 90 shows a depolarising burst in phase with the closer activity recorded in nerve 3A, while no oscillatory input is observed in the membrane potential of MN 119 (Fig. 4.6 B). In Fig. 4.6 C a wind stimulus was presented during the expression of the stridulatory motor pattern. Initially a depolarisation is seen concurrently in both motoneurons while the stridulatory motor pattern continues. After about 100 ms a second tonic excitation which generates action potentials occurs in both motoneurons following which the flight rhythm is expressed. This switching from stridulation to flight takes approximately 250 ms to complete. Recordings from an interneuron (class S-I) confirm that a central switching does in fact take place (Fig. 4.6 D). In the example shown, the oscillations in membrane potential during stridulation cease shortly after the onset of the wind stimulus. After a short lag similar to the one described above (Fig. 4.6 C), the flight rhythm is expressed and phase coupled IPSPs, together with a general inhibition, prevent this interneuron from being active during the flight rhythm (Fig. 4.6 D). The wind stimulus only induced the switching from stridulation to flight. The flight sequence lasted longer than the wind stimulation as did the inhibition and therefore the pattern of inhibition seen during flight activity was not mediated directly by sensory wind input. In general, this rapid switching was

only observed while inducing flight during ongoing stridulation (Fig. 4.6 C, D). Stridulation may follow flight activity within a few hundred milliseconds, but it rarely appeared to be able to interrupt or terminate the ongoing flight rhythm.

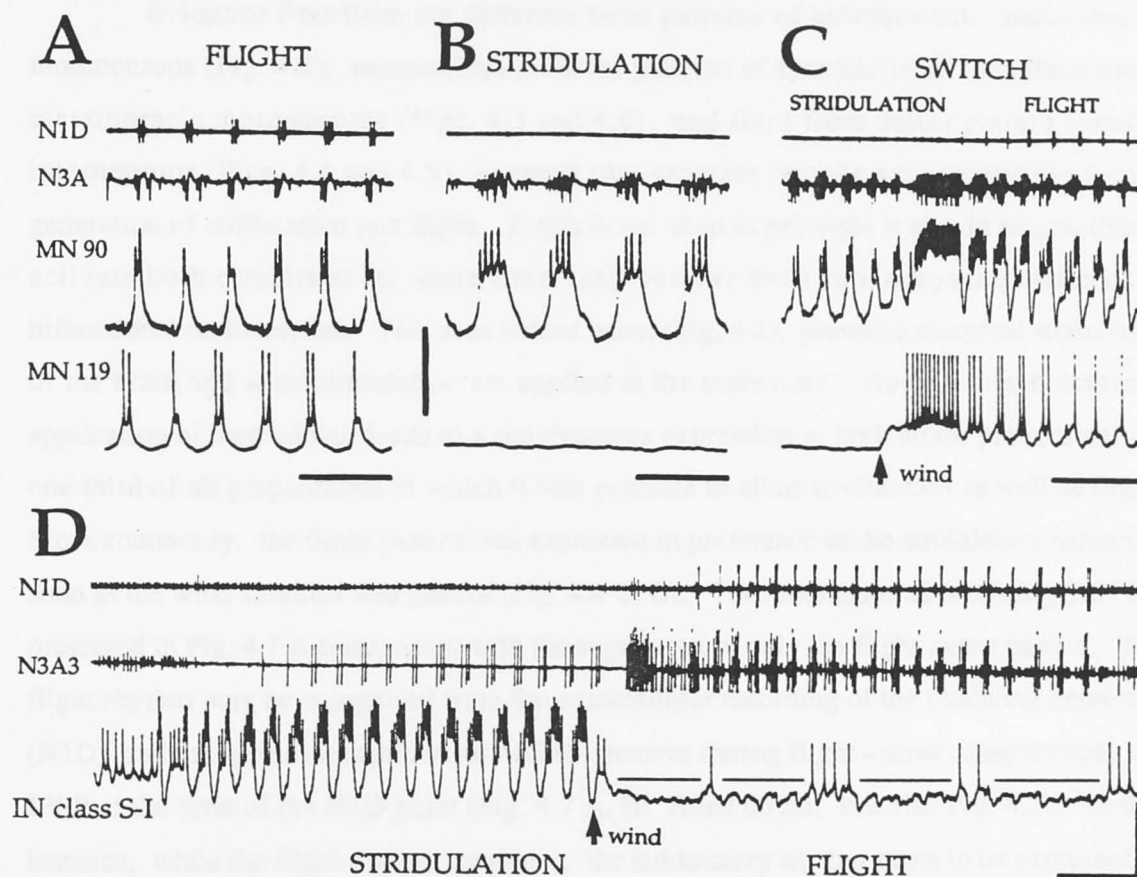


Fig. 4.6 A - D. Activity patterns of presumed serially homologous meso- and metathoracic motoneurons (MN 90 and MN 119) during stridulation and flight, as well as during switching between the two rhythms. Recording traces are as in Fig. 4.4 except that there are two intracellular traces instead of one. **A** Both motoneurons function as elevators during flight and their bursts are terminated when a depressor burst in the metathoracic dorsal longitudinal muscle (recorded from nerve 1D) occurs. **B** During stridulation MN 90 shows strong oscillations in membrane potential while MN 119 displays no phasic pattern of synaptic input. **C** These differences in neuronal activity (**A**, **B**) become very obvious when flight is induced by a wind stimulus (see arrow) during ongoing stridulation: the motor pattern expressed by the animal switches from stridulation to flight and the synaptic input pattern to both motoneurons changes accordingly. **D** The same drastic switch is seen when this unidentified interneuron, which bursts in antiphase to the closer burst during stridulation (class S-I, Chapter 2), is inhibited both by IPSPs phase coupled to the flight cycle, and by an ongoing hyperpolarisation. Line indicates resting potential. Calibration: Vertical **A** - **C** MN 90: 20 mV MN 119: 40 mV **D** 20 mV; Horizontal **A**, **B** 100 ms **C** 250 ms **D** 200 ms.

4.4. Simultaneous occurrence of stridulation and flight

Evidence first from the different burst patterns of bifunctional, mesothoracic motoneurons (Fig. 4.2), second from different patterns of synaptic input to unifunctional, metathoracic motoneurons (Figs. 4.3 and 4.6), and third from motor pattern specific interneurons (Figs. 4.4 and 4.5) suggests that separate circuits are responsible for the generation of stridulation and flight. If this is so, then in principle it should be possible to activate both circuits at the same time, and observe their superimposing patterns in bifunctional motoneurons. This does indeed occur (Fig. 4.7), provided electrical stimulation of the brain and wind stimulation are applied at the same time. However, simultaneous application of both stimuli leads to a simultaneous expression of both motor patterns only in one third of all preparations in which it was possible to elicit stridulation as well as flight. More commonly, the flight pattern was expressed in preference to the stridulatory pattern as soon as the wind stimulus was present (Fig. 4.6 C, D). The motoneuronal recording (MN 99) presented in Fig. 4.7 A commences with the expression of only the flight motor pattern. The flight rhythm may be recognised from the extracellular recording of the hindwing depressor (N1D) and the typical synaptic input MN 99 receives during flight - most conspicuously the IPSP at the time of the N1D burst (Fig. 4.7 A, B: filled circle; see also Fig. 4.2). In this instance, while the flight rhythm continues, the stridulatory rhythm starts to be expressed as well, without further stimulation, resulting in the superposition of synaptic inputs typical for both motor patterns (Fig. 4.7 A, C, D; Fig. 4.2). The fast phasic depolarisation typical of synaptic input during stridulation is clearly present (Fig. 4.7 A, C, D: triangles). Finally, flight activity ceases and the recording sequence shows only continuing stridulatory activity (Fig. 4.7 A, E). Importantly, the stridulatory cycle is not phase locked to the flight cycle, and as indicated (Fig. 4.7 C, D), the stridulatory input is equally likely to occur in the middle of a flight cycle as at the end. The phase histograms for both cases (stridulation in a flight cycle (*str in fli*) and flight during a stridulatory cycle (*fli in str*); Fig. 4.8) show that there is no strict phase coupling between the two, simultaneously occurring, motor patterns.

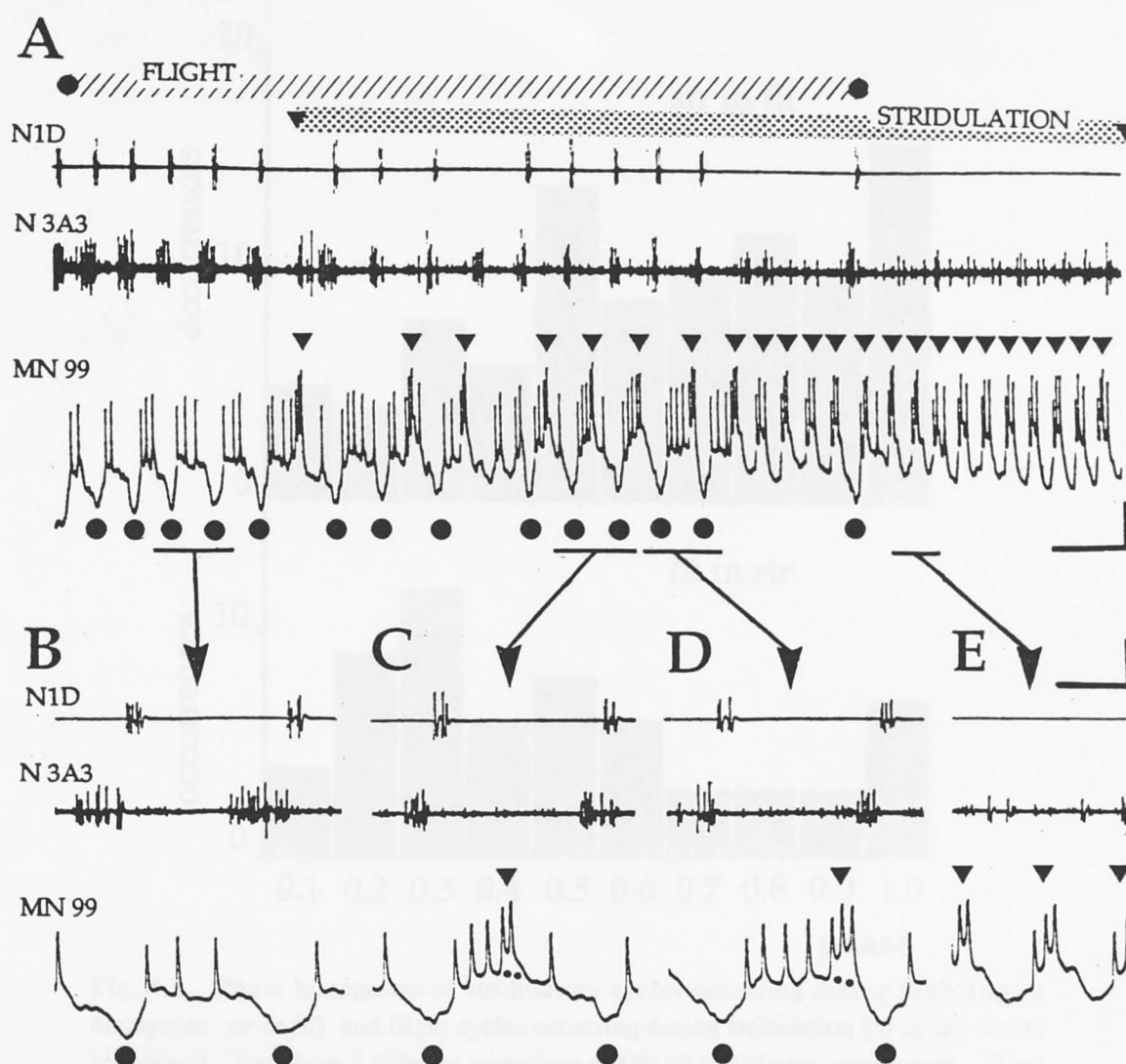


Fig. 4.7 A - E. The motor patterns for flight and stridulation may be expressed simultaneously, if electrical and wind stimulation are applied at the same time. The recording shown is taken from motoneuron 99 (c.f. Fig. 4.2 for a comparison of the synaptic input to this motoneuron during the expression of the two motor patterns in isolation; upper trace: metathoracic nerve 1D; middle trace: mesothoracic nerve 3A3; lower trace: intracellular recording of MN 99). **A** Simultaneous expression of flight and stridulatory activity in a continuous recording of motoneuron 99. Initially only the flight motor pattern is expressed as is apparent from the rhythmical activity in nerve 1D, and the typical burst pattern in the intracellular recording of MN 99 (IPSPs at the time of nerve 1D bursts are marked by a filled circle). Then the stridulatory motor pattern becomes superimposed on the flight motor pattern, as may be recognised from the typical phasic bursts (marked by filled triangles) which occur exclusively during stridulation in this motoneuron. The sequence ends with expression of the stridulatory rhythm alone after cessation of the flight rhythm (lack of bursting activity in the recording of metathoracic nerve 1D). **B, C, D, E** Enlarged bursts from selected sections of the recording in **A** (filled circle: IPSP at the time of N1D discharge (flight depressor); triangle: phasic stridulatory burst). **B** An undisturbed flight cycle. **C** A section with flight and stridulatory inputs to MN 99. The stridulatory burst occurs half-way during the flight cycle. The normal flight input is indicated by the dotted line. **D** The stridulatory burst occurs at the end of the flight cycle. **E** An undisturbed stridulatory cycle. Calibration: Vertical **A - E** 20 mV; Horizontal **A** 100 ms **B - E** 25 ms.

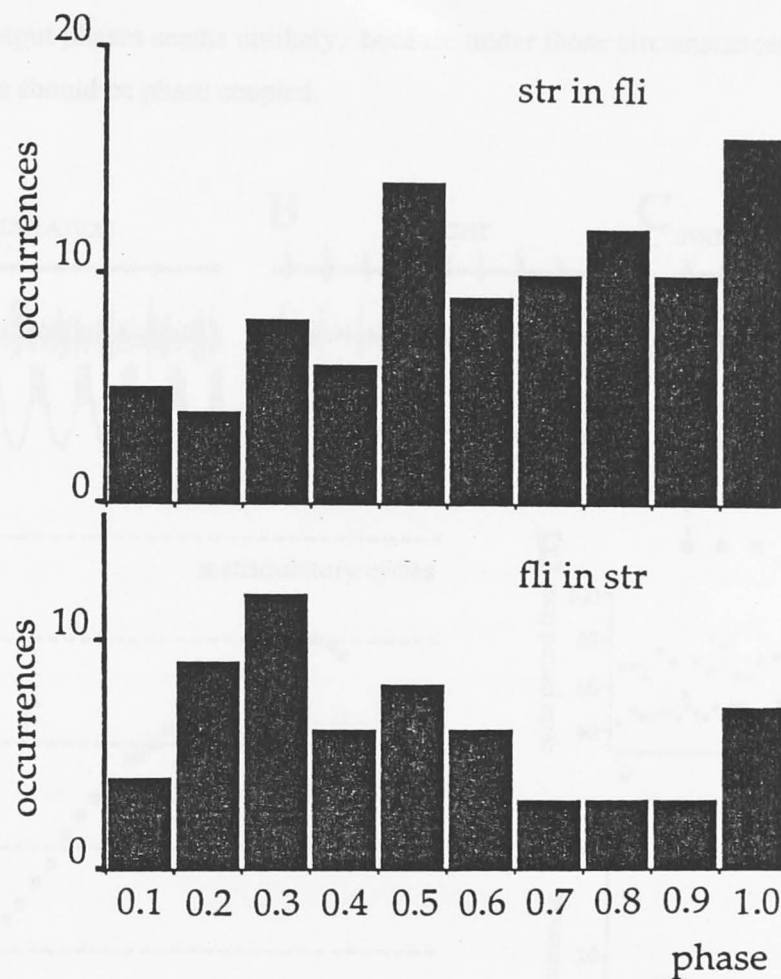


Fig. 4.8. Phase histograms of stridulatory cycles occurring during flight (upper histogram: *str in fli*) and flight cycles occurring during stridulation (*fli in str*: lower histogram). Data from 7 different recordings of MN 99 in different preparations. There is no strict phase-locking between the cycles of stridulation and flight.

In instances where both motor patterns occurred simultaneously, each pattern often appeared to become irregular compared to its expression in isolation. This may be due to the inhibition seen in interneuronal recordings (Figs. 4.4 and 4.5). There are, however, also instances where both motor patterns run undisturbed and at normal cycle durations (Fig. 4.9). The example shown stems from a stridulatory interneuron (Fig. 4.9 A; class S-I, chapter 2) which receives a barrage of IPSPs during flight (Fig. 4.9 B). When both motor patterns are evoked simultaneously (Fig. 4.9 C), they drift past each other for almost 40 flight cycles undisturbed (Fig. 4.9 D) and the cycle durations for each rhythm are within the normal range as expressed in isolation (Fig. 4.9 E). The phase histogram for stridulatory cycles in a flight cycle also does not suggest any coupling between the two rhythms (Fig. 4.9 F). Thus, the possibility of a single central oscillator generating two rhythms at the same time by using

different output phases seems unlikely, because under those circumstances the cycles of the two rhythms should be phase coupled.

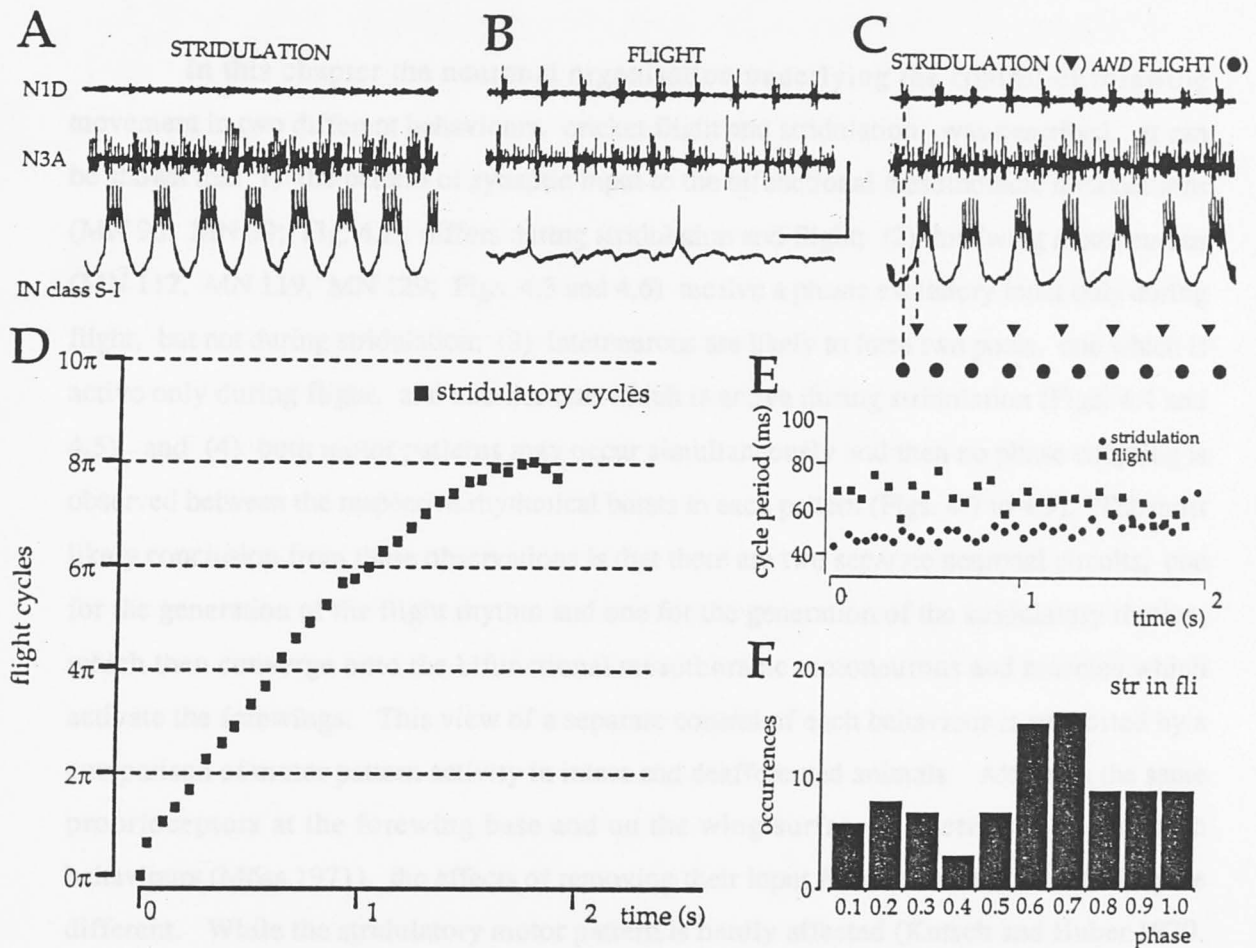


Fig. 4.9 A - F. Simultaneous occurrence of stridulation and flight in a stridulatory interneuron (class S-I, Chapter 2) evoked by simultaneous electrical and wind stimulation. **A** The activity of this interneuron during stridulation shows that the bursts are coupled to the opener phase. **B** During flight, activity in this interneuron is normally suppressed by IPSPs. **C** In this preparation it was possible to induce flight and stridulation simultaneously. Under these circumstances the motor patterns drift past each other without any apparent phase locking (flight bursts are marked with a filled circle, stridulatory bursts are marked with a black triangle). **D** Plotting the stridulatory bursts against the phases of the flight cycles shows that the stridulatory rhythm is not coupled to the flight rhythm. The drift ceases because the flight rhythm slows down and ends. **E** Under these conditions both the flight pattern and the stridulatory pattern are expressed with their normal cycle durations (i.e. stridulation: ~63 ms, flight: ~51 ms). This observation was made more than 5 times in this and also several other preparations, but only here both rhythms occurred simultaneously for a longer period in time (i.e. more than 1 s). **F** A plot of phase occurrences of stridulatory bursts during the flight cycles shows that stridulatory bursts may occur at any phase during a flight cycle. Calibration: A - C Vertical 10 mV; Horizontal 100 ms.

5. DISCUSSION

5.1. *Evidence for a separation of networks*

In this chapter the neuronal organisation underlying the control of forewing movement in two different behaviours, cricket flight and stridulation, was described. It can be shown that 1) the pattern of synaptic input to the bifunctional mesothoracic motoneurons (MN 90, MN 99; Fig. 4.2) differs during stridulation and flight; (2) hindwing motoneurons (MN 112, MN 119, MN 129; Figs. 4.3 and 4.6) receive a phasic excitatory input only during flight, but not during stridulation; (3) interneurons are likely to form two pools, one which is active only during flight, and another one which is active during stridulation (Figs. 4.4 and 4.5); and (4) both motor patterns may occur simultaneously and then no phase coupling is observed between the respective rhythmical bursts in each pattern (Figs. 4.7 to 4.9). The most likely conclusion from these observations is that there are two separate neuronal circuits, one for the generation of the flight rhythm and one for the generation of the stridulatory rhythm, which then converge onto the bifunctional mesothoracic motoneurons and muscles which activate the forewings. This view of a separate control of each behaviour is supported by a comparison of motor pattern activity in intact and deafferented animals. Although the same proprioceptors at the forewing base and on the wing surface are activated during both behaviours (Möss 1971), the effects of removing their input from the central circuits is quite different. While the stridulatory motor pattern is hardly affected (Kutsch and Huber 1970, Möss 1971, Schöffner and Koch 1987, Chapter 2), the flight motor pattern shows clear changes in cycle duration and in the phase relationships between antagonistic muscles (Robertson 1987, Chapter 3). Conclusive evidence on the separation of the networks generating the two motor patterns still requires the demonstration of separate functions for initiating and resetter interneurons. Nevertheless, there are phase coupled, as well as tonic, inhibitory interactions between the two systems. This observation suggests that there are synaptic connections at both the initiating and the pattern generating level between the two circuits. These connections appear to ensure that only one motor pattern is executed at a time since there appears to be no adaptive value in the simultaneous occurrence of both behaviours.

5.2. Evolutionary aspects of cricket flight and stridulation

Previous work on the flight and stridulatory motor patterns in the cricket (Huber 1962, Kutsch 1969) suggested that both behaviours are driven by a common central oscillator. Based on EMGs obtained from *Gryllus campestris*, whose stridulatory behaviour may be described as short flight sequences (chirps; Huber 1962), these findings supported the view that flight and stridulation are related behaviourally and neuronally, and that they also share a common evolutionary origin (Zeuner 1939, Huber 1962, Alexander 1962). Although the findings in this present study do not support the idea of a shared neuronal oscillator between the two motor patterns, they cannot disprove that this may originally have been the case. The complex stridulation pattern of *T. commodus* is quite different from the flight pattern, particularly with respect to the switching between the chirp and trill rhythms (Bentley and Hoy 1972, Chapter 2). Intraspecific communication requires this accurate timing of the syllables to maintain the species isolating function of the song. Selection pressure may therefore have favoured a separate control circuitry in order to execute both motor patterns as accurately as possible. A comparative study of the neuronal basis of sound production in crickets with different song patterns is needed to better understand this evolutionary aspect.

5.3. Comparison with other systems

Some of the present findings are similar to those of other investigations of the use of the same limb or appendage during the execution of different behaviours. For instance, (1) motoneurons innervating bifunctional muscles may receive synaptic input during both of the behaviours in which these muscles are involved (Simmers and Bush 1983, Heitler 1985, see also Elsner 1974, Ramirez and Pearson 1988). (2) Different patterns of proprioceptive feedback appear to be responsible for the modification of a common central rhythm and the production of different behaviours in some systems (Ayers and Davis 1977, Reingold and Camhi 1977, Sherman et al. 1977, Zill 1986, Koshland and Smith 1989, Bekoff et al. 1987). This differs from cricket stridulation and flight where information from the same sets of proprioceptors is most likely fed to different networks. (3) Separate neuronal networks were also proposed to converge onto the same appendage in crustaceans after EMG and behavioural analyses showed cyclical movements in exopodite and endopodite of the same appendage. Different neuronal oscillators were presumed to generate these cyclical movements because of

the weak or absent coupling between them (Laverack et al. 1977, MacMillan et al. 1981).

(4) Based on EMG analysis of multifunctional appendages, several studies have concluded that the same motor program may underlie different behaviours (see table 4.1: Kammer 1968, Ayers and Davis 1977, Reingold and Camhi 1977, Sherman et al. 1977, Ayers and Clarac 1978, Pflüger and Burrows 1978, Simmers and Bush 1983). However, intracellular analysis of the swimmeret system in the crayfish (Heitler 1985) and the motor patterns activating bifunctional muscles during flight and walking in the locust (Ramirez and Pearson 1988) have demonstrated that there may equally be a separation of neuronal networks controlling these behaviours. The motor patterns in the latter two instances differed markedly in the magnitude of parameters such as cycle periods and burst amplitudes, in contrast to other motor programs activating the same appendage. Ramirez and Pearson (1988, see also Gramoll (1988) for acridid stridulation and flight) also reported interneurons which were specific for the expression of only one motor pattern. Although the cycle periods during stridulation and flight in the cricket *Teleogryllus commodus* are similar, other characteristics of the motor pattern, such as the switching between the chirp and the trill rhythms during stridulation, are quite different from anything seen during flight. Additionally, a separation of interneurons involved in each behaviour was also found in the present study. Investigations of vertebrate limb movements involved in different behaviours generally support the idea of shared neuronal networks (Berkinblit et al. 1978, Carter and Smith 1986, Robertson et al. 1985, Bekoff 1986). These are now commonly referred to as polymorphic (or multifunctional) networks (Getting and Dekin 1985) and they allow the nervous system to adjust for the production of a range of motor patterns in a versatile and economical manner. On the other hand, the proper execution of the motor programs investigated in the present study requires accurate timing, very stereotyped movement, no intermediate forms, and a clear separation in time. These factors may require the implementation of different neuronal networks to control the same appendage in different behaviours.

CHAPTER V

GENERAL DISCUSSION

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This study has examined the neural basis for stridulation and flight in the cricket, *Teleogryllus commodus*, and has presented evidence that separate pools of interneurons control these two behaviours. In this chapter I will discuss the similarities and differences in the motor pattern generation for each behaviour and relate the findings presented in the previous chapters to the broader principles of motor pattern generation.

1. *Comparative aspects of motor pattern generation for stridulation and flight.*

Stridulation and flight in the cricket both involve use of the wings and employ the thoracic musculature under the control of the thoracic ganglia. What are the similarities and the differences in the neuronal control over these two motor patterns? The calling song in crickets acts as an interspecific pre-mating isolating mechanism and to perform this role most effectively must differ from the songs of other species. Flight behaviour, on the other hand, is likely to be quite similar in all flying crickets. This implies that species-specific differences are likely to exist in the motor patterns generated for stridulation but not those generated for flight. Because of their different functions, the selection pressures on each system may also have been different, thus resulting in different mechanisms of motor pattern generation.

(1) *Central versus peripheral control*: Most animal behaviour may be described as a closed-loop system in that the organism obtains feedback information about its own movement while a motor act is executed. Only a few behaviours, such as the strike behaviours of predators (Mittelstaedt 1964, Messenger 1968, Ewert 1980) function under open-loop conditions where the control system does not receive feedback during completion of the motor act. During both stridulation and flight the nervous system of the cricket receives phasic feedback on a cycle-by-cycle basis from proprioceptors at the wing base and this peripheral

feedback is crucial for successful generation of the behaviours (Möss 1971, Schöffner and Koch 1987). However, during stridulation the timing of motor bursts (i.e. the motor pattern) is largely independent of sensory feedback (Kutsch and Huber 1970, Elliott and Koch 1983, Schöffner and Koch 1987, Chapter 2), whereas during flight, peripheral information has a major influence on the timing of motor bursts (Robertson 1987, Chapter 3). This different weighting of peripheral information during pattern generation results in the trill rhythm (Figs. 2.2 and 2.3) and the flight rhythm (Fig. 3.1) having similar cycle periods (i.e. ~ 30 Hz), but this frequency is achieved by largely central mechanisms during stridulation, and by the integration of central and peripheral mechanisms during flight. The difference in the role of sensory input in the two behaviours may be related to their different functions. During flight, the cricket has to take account of wind perturbations and negotiate obstacles within a few wing beat cycles. During stridulation, the cricket does not have to take account of any external disturbances but mainly needs to adjust for proper wing alignment. A wind or any other environmental perturbation during stridulation may signal the approach of a predator and it will generally be selectively advantageous for the cricket to stop singing and to escape. By contrast, the accurate timing of syllables required during stridulation may be more successfully achieved by central mechanisms independent of perturbation. Different selection pressures may therefore have favoured a different mechanism for processing sensory information in each behaviour.

(2) *Phase characteristics in a cycle*: A description of both the phase relationships between bursts in antagonistically acting muscles, and how these phase relationships change at varying cycle frequencies of the motor pattern, are important for understanding the neuronal control of behaviour. During stridulation and flight in the cricket the phases of activity of antagonistic muscles remain approximately constant (Fig. 2.2, Robertson 1987). The cycles in each motor pattern are unevenly divided (so that one interval in a biphasic rhythm is longer than the other) in intact as well as deafferented animals (Figs. 2.2, 2.3, 3.6; Robertson 1987). Importantly, after deafferentation, the power stroke of each behaviour (i.e. closing during stridulation and depression during flight) is essentially time locked to the return stroke activity (Fig. 2.3, Robertson 1987). However, the power strokes of stridulation and flight do not involve the same muscles: muscle 90 conducts the power stroke during stridulation and the return stroke during flight; whereas muscle 99 conducts the power stroke during flight, but the return stroke during stridulation (Fig. 1.1). Thus, the time-locking of power to return stroke involves the same muscles but in reverse order during each motor pattern. This is an

important difference between the neuronal mechanisms which generate the stridulatory and flight motor patterns in deafferented animals.

(3) *Command structures*: Rhythmical motor patterns are commonly initiated by so-called command systems (Chapter 1: 1.3.). There is evidence that such command systems exist in both stridulation and flight (Bentley 1977 and Fig. 3.11) and that these systems are similar, in that they show gating properties (Chapter 1: 1.3., Bentley 1977) and descend to the motor centers in the thoracic cord. At present, it appears likely that each motor pattern is initiated by its own command system, since stridulation and flight cannot be elicited from the same brain regions (Huber 1960a), and Bentley (1977) also induced only stridulation while stimulating small groups of ventral cord neurons. Furthermore, the likely candidates for flight initiation (T404) and their counterparts in the locust (404) are most sensitive to wind, which is not an initiating stimulus for stridulation (Pearson et al. 1985, Ramirez 1988). The command systems for stridulation and flight in the cricket are likely to consist of a number of neurons all of which contribute to the initiation of the motor pattern, however, conclusive evidence by recordings from several identified cells is not yet available. On the other hand, there are some observations which indicate marked differences between the initiation of stridulation and of flight. Firstly, changes in the discharge frequency of putative command neurons during flight influence the flight frequency (Fig. 3.11), whereas similar changes in command neurons for stridulation influence the repetition rate of chirps rather than pulses (Bentley 1977), as might be predicted if the two systems were similarly organised. Stimulation so as to produce more pronounced changes in the discharge rate of descending neurons can even evoke different song patterns (Bentley 1977). Thus, the information encoded in the discharge frequency by apparently comparable interneuron(s) may have quite different effects on different motor systems. However, it is conceivable that flight frequency and chirp rate are neuronal analogues (Huber 1962, Elsner 1983), which would support the idea of evolutionary links between the two networks (Chapter 4). Secondly, the conversion of the tonic discharge pattern produced by gating neurons (Bentley 1977, Fig. 3.11) into the rhythmical burst pattern required for behaviour seems to follow different principles in stridulation and flight. During stridulation the excitatory drive appears to be weak initially and this results in only subthreshold oscillations in the motoneurons (Figs. 2.1 and 2.7). While the strength of this drive slowly increases, the stridulatory cycle period stays almost constant (Fig. 2.9). The activity pattern of some of the stridulatory interneurons correlates with the subthreshold activity seen in motoneurons (Fig. 2.9 C), but other interneurons show

the full burst pattern while motoneuronal activity is still subthreshold (Figs. 4.6 D). During flight, on the other hand, there is a distinct initial depolarising phase which precedes the expression of the motor pattern (Figs. 3.4, 3.5, 4.6). As the flight rhythm continues, the excitatory drive and the motor pattern frequency both decrease in the absence of wind, as recorded in deafferented preparations (Figs. 3.4, 3.5, 3.11). Concomitant changes may be seen in motoneuronal and interneuronal burst patterns alike (Figs. 3.4, 3.5, 3.11). Thus, although both motor pattern generators appear to use a gating system for the initiation of the behaviour, the mechanism of conversion of tonic neuronal activity to rhythmical activity by the respective motor generator system appears to be quite different in each case.

(4) *Action potential induced versus graded synaptic transmission:* The mechanisms of motor pattern generation in many invertebrates involve neurons generating action potentials as well as neurons with a graded transmitter release (Burrows and Siegler 1978, Wilson and Phillips 1982, Graubard et al. 1983). However, non-spiking interneurons have not yet been unequivocally demonstrated in the stridulatory or flight system of the cricket, nor the flight system of the locust. On the other hand, some of the mesothoracic muscles employed during stridulation and flight in the cricket also serve to move the leg. During walking, non-spiking interneurons appear to play important roles in the generation of the central rhythm (Pearson and Fournier 1975), as well as in the integration of sensory information transmitted to motoneurons essential for the intersegmental coordination of the locust leg during stepping (Burrows and Siegler 1978, Burrows 1980). Assuming there is indeed a neuronal separation of the walking and the flight systems of the locust as suggested by Ramirez and Pearson (1988), a similar situation is also likely to exist in the cricket. This implies that the ventral nerve cord of the cricket may possess up to three networks which activate some of the mesothoracic muscles during three different behaviours. These behaviours not only differ in cycle period, but also in the phase relationships of bursts of antagonistic muscles, in changes of synergistic and antagonistic burst patterns, and possibly in the type of synaptic interaction between neurons.

2. Aspects of shared motor control

This investigation presents evidence that the forewings and associated muscles of the cricket are activated by different neuronal circuits during the generation of the stridulatory and flight motor patterns (Chapter 4). Although the neuronal circuits involved have not been

identified in all their details, indirect supporting evidence for this conclusion comes from (1) the different effects of deafferentation on the two behaviours, (2) the different coupling of power and return strokes in a cycle, and (3) the different characteristics of the command system. How does this proposal of a separate neuronal control for the same muscles and appendage in the cricket relate to previous studies on motor patterns which use the same muscles in different behaviours in other organisms? Table 5.1 lists some selected studies concerned with this question of neuronal organisation. A distinction is made between motor patterns involving the same appendage (limb) and those involving the same muscles. These two groups are each subdivided into examples from invertebrates and vertebrates. For each of these studies the method of analysis (EMG, intracellular from motoneurons (MN) or interneurons (IN)), the mechanisms of switching between motor patterns (central (apparently spontaneous), sensory), and the proposed form of neuronal organisation (multifunctional (MF), separate) is also indicated.

(1) *Switching between motor patterns*: Most studies report a switching between motor patterns in response to an external sensory stimulus (table 5.1: [2, 6, 9, 10, 11, 14, 16, 19, 20, 21, 24]) as found in this study with the switching from stridulation to flight. The stomatogastric system [18] is an exception, since neurotransmitters evoke the changes in the motor rhythm. However, the changes in neurotransmitter concentration must ultimately depend on the type of food being digested. In only a few cases does switching appear to occur spontaneously without any apparent external sensory stimulation [1, 4, 5, 17]. This apparently stimulus independent switching was also found in the switching from chirp to trill during stridulation.

What are the proposed mechanisms for switching between motor patterns? In cases of external sensory stimulation it is usually the same set of sensory axons which carry the different information (commonly a brief and weak as opposed to a strong and prolonged stimulus) centrally to interneurons which then, depending on their pattern of activity, induce changes in motor pattern expression [16, 19, 20, 21, 23, 24, 27]. In some cases motoneurons are affected directly by sensory stimulation [16, 20]. In cases of apparent spontaneous switching, both interneurons [1, 17] and motoneurons [4] may initiate the change in motor pattern. The mechanism switching the chirp and trill rhythms in the cricket is likely to reside at an interneuronal level (Fig. 2.4), however, conclusive evidence for this is not yet available.

Table 5.1: Descriptions of different motor patterns involving the same appendage (limb) [1 - 12] and muscles [13 - 24].

<i>Appendage movements - Invertebrates</i>						
<i>animal</i>	<i>appendage</i>	<i>behaviours</i>	<i>analysis</i>	<i>switching</i>	<i>organisation</i>	<i>reference</i>
[1] crayfish	swimmeret	two motor patterns	IN	central, spont.	separate	Heitler (1985)
[2] cockroach	leg	walking, righting	EMG	sensory	MF	Sherman et al. (1977), Camhi (1977) Reingold and Camhi (1977), Zill (1986)
[3] lepidoptera	wings	flight, warm-up	EMG	central	MF	Kammer (1968)
[4] crab	scaphognathite	forward, reverse beating	EMG, MN	central, spont. MNs	MF	Simmers and Bush (1983)
[5] <i>Limulus</i>	gills	cleaning, ventilation swimming	EMG	sens., central	separate	Watson (1980a, 1980b)
[6] locust	hindleg	swimming, jump, kick	EMG	sensory	MF	Pflüger and Burrows (1978)
[7] lobster	leg	walking in various directions	EMG	?	MF	Ayers and Clarac (1978) Ayers and Davis (1977)
[8] cricket	forewings	stridulation and flight	EMG	sensory & central	MF	Huber (1962), Kutsch (1969)
<i>Limb movements - Vertebrates</i>						
<i>animal</i>	<i>limb</i>	<i>behaviours</i>	<i>analysis</i>	<i>switching</i>	<i>organisation</i>	<i>reference</i>
[9] cat	leg	stepping and scratching	IN, EMG	sensory	MF	Berkinblit et al. (1978)
[10] cat	leg	walking and paw-shake	EMG	sensory	MF	Carter and Smith (1986) Smith et al. (1986 in Goldberger)
[11] turtle	leg	three scratch patterns	EMG, MN	sensory	MF	Robertson et al. (1985) Robertson and Stein (1988) Currie and Stein (1989)
[12] chicken	leg	walking and hatching	EMG	?	MF	Bekoff (1986), Bekoff et al. (1987)

Shared muscles - Invertebrates

<i>animal</i>	<i>muscle</i>	<i>behaviours</i>	<i>analysis</i>	<i>switching</i>	<i>organisation</i>	<i>reference</i>
[13] crayfish	abdominal	tonic and cyclical	IN	?	separate	Moore and Latimer (1988)
[14] locust	leg muscles	walking and flight	IN, MN, EMG	sensory	separate	Ramirez and Pearson (1988)
[15] grasshopper	leg muscles	stridulation and flight	EMG	sens., central	MF	Elsner (1974, 1983)
[16] leech	body muscles	shortening, crawl, swim	IN	sensory	MF	Kristan et al. (1988)
[17] leech	heart	peristaltic and non-peristaltic modes	IN	central, spont.	MF	Calabrese (1979)
[18] lobster	stomatogastric	different rhythms	IN	neuromodul.	MF	Marder et al. (1987) Hooper and Moulins (1989) Dickinson et al (1990) Selverston (1988)
[19] molluscs	radula	egestion, ingestion	IN, EMG	sensory, INs	MF	Croll and Davis (1987)
[20] <i>Aplysia</i>	gill, siphon	movements and reflexes	IN, MN	sens. INs, MNs	MF	Kupfermann et al. (1974)
[21] <i>Tritonia</i>	veil	withdrawal and swim	IN	sensory	MF	Getting and Dekin (1985) Getting (1989a)
[22] cricket	all muscles	eclosion behaviour	EMG	sens., centr.		Carlson (1977)

Shared muscles - Vertebrates

<i>animal</i>	<i>muscle</i>	<i>behaviours</i>	<i>analysis</i>	<i>switching</i>	<i>organisation</i>	<i>reference</i>
[23] electr. fish	electric organ	JAR and chirps	IN	sens., central	separate	Keller and Heiligenberg (1989), Kawasaki and Heiligenberg (1988, 1989), Kawasaki et al. (1988), Dye et al. (1989)
[24] <i>Xenopus</i>	body muscles	swim and struggle	EMG	sensory	?	Roberts et al. (1983) (see also Kahn and Roberts 1982a, b)

(2) *Separate or shared neuronal organisation:* The cellular investigations of the neuronal organisation underlying the different motor patterns listed in table 5.1 have revealed networks organised in three different ways: (a) the same network of neurons generates various motor patterns as a result of changes in synaptic strength and neuronal burst patterns [17, 18]; (b) a network of neurons generates different motor patterns by a reconfiguration of the cellular circuit which results from different strengths of sensory stimulation [16, 20, 21]; and (c) separate networks are responsible for the generation of the motor patterns [1, 13, 14] as is also suggested by the present study. The first two forms of organisation (a and b) are also referred to as multifunctional or polymorphic networks (Getting and Dekin 1985, Getting 1989a). Criteria to distinguish between these organisations include factors such as whether both motor patterns can occur simultaneously. This distinction is not always useful. For instance, in electric fish the oscillator activity generating the regular electric organ discharge and chirping during courtship arises independently from different centres in the nervous system (Kawasaki et al. 1989). These two motor rhythms may also occur simultaneously with only little interference, indicating a separation and independence of the neuronal oscillators (Dye et al. 1989, Kawasaki and Heiligenberg 1989, Heiligenberg 1989). However, both motor patterns share sensory receptors (Dye 1987), as well as the relay cells in the pacemaker nucleus (Kawasaki et al. 1988), and may thus be considered as a multifunctional network. Similarly, the rhythm and pattern generators for stridulation and flight in the cricket appear to be independently organised. There are, however, also concomitant changes in neural activity associated with both behaviours such as changes in respiratory activity, leg position, and abdominal posture. It seems unlikely that these linked changes in motor pattern generation would be driven by three separate independent circuits exclusive for either stridulation or flight.

In many cases the proposal of a multifunctional or separate network is based solely on EMG analysis of the motor patterns (table 5.1; [2, 3, 5, 6, 10, 11, 15]). Common criteria by which the motor patterns are compared in these analyses are the cycle periods, the phase relationship of antagonistic bursts in a cycle, and the burst amplitudes. A common conclusion is that if these parameters are different between the two motor patterns then this implies the presence of separate networks [1, 5] as is also supported by other intracellular investigations [13, 14]. If on the other hand, those parameters are similar then a multifunctional network is proposed [2, 3, 4, 6, 10, 11, 15] as is supported by some intracellular investigations [4, 19]. However, these proposals need to be substantiated by cellular investigations, since in some

cases quite drastic differences in the parameters characterising a motor pattern result from a multifunctional network [18, 21], whereas separate networks may generate similar cycle durations and burst amplitudes as shown in this study.

The results obtained in this study suggest that the motor pattern generation for stridulation and flight in the cricket may be based on different principles. An important step towards an understanding of these differences on a cellular level has now been taken, since the motor patterns have been described, techniques to elicit both behaviours have been developed, and the first likely cellular candidates for rhythm initiation and rhythm generation have been identified. Further investigation is needed to establish the command structure for each system, to elucidate the basis for the central rhythm, and to gain insight into the cellular integration of peripheral feedback. The cricket is certainly well suited to advance our knowledge in these areas, but also promises to be a very fruitful preparation for gaining some insight into (1) how the separate circuits for stridulation and flight interact on a cellular level so that only one motor pattern is generated at a time, (2) how the nervous system controls and generates the different song patterns of an individual male cricket (calling song, aggressive song, courtship song) where it is quite conceivable that many neurons are shared, and (3) how the neuronal networks for different calling song patterns in different cricket species are organised and thus how a possibly "ancestral" network might change during evolution in order to generate different motor patterns (Dumont and Robertson 1986).

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